
Lighting Requirements in Microgravity – Rodents and Nonhuman Primates

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Lighting Requirements in Microgravity – Rodents and Nonhuman Primates

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DEDICATION OF WORKSHOP TO PROFESSOR CURT P. RICHTER
THE JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE

The Space Life Sciences Payloads Office and the Department of Biological Sciences, San Jose State University, dedicate these proceedings to Professor Curt P. Richter, of The Johns Hopkins University Medical School.

Dr. Richter's work in animal behavior and physiology has spanned over 60 years (see Richter, C. P.: A behavioristic study of the activity of the rat. *Comp. Psychol. Mongr.*, vol. 1, 1922, pp. 1-55; and Richter, C. P.: Growth hormone 3.6-hr pulsatile secretion and feeding times have similar periods in rats. *Am. J. Physiol.*, vol. 239 [Endocrinol. Metab.], 1983, pp. E1-E2). He has done research in his laboratory and published on many of the topics covered in this volume. These creative and philosophical contributions have significantly and immeasurably influenced several fields of scientific investigation and will do so for years to come. Richter, C. P.: Biological Clocks in Medicine and Psychiatry. Charles C. Thomas Publisher, 1965, continues to be one of the most valued early reviews on the subject. It is fitting, as we gather to define the limits and standards of the artificial animal environments to be used in space, that we acknowledge and pay our respects to one who has done so much to enhance our functional understanding of the animal body and mind.

ACKNOWLEDGMENTS

The editors gratefully acknowledge Ms. Kara M. Myers, Angela C. Tischler, and Karen Rossberg for technical support and making necessary arrangements for this important meeting. We also appreciate the excellent help provided by Ms. Kara M. Myers, Cynthia L. Race, and Lisa M. Gibbs in preparation of this manuscript.

Daniel C. Holley, Ph.D.
Charles M. Winget, Ph.D.
Henry A. Leon, Ph.D.

Note: All credits for illustrations appear together in Appendix B.

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BACKGROUND, SIGNIFICANCE, HISTORICAL PERSPECTIVE

A workshop, sponsored by Ames Research Center, was held at San Jose State University, San Jose, California, July 16-17, 1987, to discuss and correlate observations and theories relating to lighting requirements in animal habitats for rodents and nonhuman primates in microgravity (near space). This volume represents the results of that meeting, which was held under the auspices of the Space Life Sciences Payloads Office, Ames Research Center, and supported by the National Aeronautics and Space Administration Joint Research Interchange Number NCA2-203.

The primary objective of the meeting was to review various aspects of photobiology as they relate to rodents and nonhuman primates in the space flight environment and to apply this information to the design of current and planned space habitats for these animals.

A considerable number of space flights, both U.S. and Soviet, have included rodents and non-human primates (see table 1). As a result of the inevitable decision to continue live animal experimentation in space, NASA has been faced with the non-trivial task of developing adequate animal housing environments (habitats). Because several design teams and multiple projects have been involved in this endeavor (see Leon), it was deemed necessary to establish common environmental standards that would apply to all of the habitats.

It is well known and generally accepted that light is the most important variable in the laboratory environment for cuing the central circadian "clock" and, therefore, providing timing synchronization for literally all of the body systems. Essentially all animal facilities and research laboratories today use artificial light sources with the assumption that the light intensities and inherent spectral energies are nominal in terms of homeostasis, physiology, and behavior. However, as our understanding of the nature of light and how it interacts with living organisms expands, it is becoming apparent that light may be the single laboratory environmental factor to show greatest variation from one laboratory to another, and from one space flight to another. Inherent in good scientific investigation and methodology utilizing animals is a strict adherence to defined environmental and dietary conditions. Given that most scientific papers, and even U.S. Government laboratory animal guidelines, report light in psychometric units that relate to humans (i.e., illuminance: phot, foot-candles, or lux; or luminance: candela or lambert) it is questionable that truly reproducible light conditions can be realized from these data alone.

Understanding of the effects of light on animals in flight experiments is of particular importance to the Space Life Sciences Payloads Office because that office is ultimately responsible for the validity and quality of the experiments to be flown in microgravity. It was felt that the interactive participation of microgravity engineers, space biologists, lighting engineers, and photobiologists would be the most productive means to arrive at valid answers to the questions pertaining to lighting in the animal habitats, and would facilitate development of valid and workable lighting standards for the future.

The diversity of approaches in the study of light (as it relates to animals) and the scope of the problem necessitated the scheduling of a number of focused papers. Both current unpublished research and more inclusive review papers were considered important in giving a panoramic view of the topic of light as it relates to microgravity habitats. After deliberation it was decided not to include all extemporaneous remarks, but to encourage those who wished to have their ideas published to submit refined manuscripts. Some short connotations are included as a result.

The first paper deals with the various animal habitats current and planned, and includes a discussion of engineering limitations. The second paper deals with characterization and measurement of light. (Light was initially discussed from a physical point of view as it relates to biological applications in microgravity.) The next group of papers deals chiefly with biological timing mechanisms. The final group presents comparative aspects including animal performance and behavior, reproduction and development, and immunological considerations.

TABLE 1.- PARTIAL LIST OF RODENT AND NONHUMAN PRIMATE BALLISTIC* AND ORBITAL FLIGHTS†

| Species (N) | Mission/country | Date launch | Altitude | Duration |
|--|---------------------|-------------|----------------|-------------|
| Rhesus monkey | V2 Rocket/US | 06/11/48 | *to 62 km | |
| Rhesus monkey | Blossom 3/US | 06/18/48 | *to 62 km | |
| Rhesus monkey | Blossom 4/US | 10/00/48 | *to 134 km | |
| Cynomolgus monkey | Blossom 5/US | 05/00/49 | * | |
| Cynomolgus monkey | Blossom 6/US | 05/00/49 | * | |
| Rhesus monkey | V2 Rocket/US | 06/14/49 | *to 134 km | |
| Cynomolgus monkey | V2 Rocket/US | 09/16/49 | *to 35,000 ft | |
| Cynomolgus monkey | V2 Rocket/US | 12/08/49 | *to 200,000 ft | |
| Mouse | Blossom 7/US | 07/00/50 | * | |
| Mouse | V2 Rocket/US | 10/31/50 | *to 136 km | |
| Cebus monkey, mice | Aerobee Rocket/US | 04/18/51 | *to 61 km | |
| Rhesus monkey, mice (11) | Aerobee Rocket/US | 09/20/51 | *to 71 km | |
| Cebus monkeys (2), mice (2) | Aerobee Rocket/US | 05/21/52 | *to 62 km | |
| Mouse | Thor IRBM/US | 04/23/58 | *to 1400 mi | 20 min |
| Mouse | Thor IRBM/US | 07/09/58 | *to 1400 mi | 20 min |
| Mouse | Thor IRBM/US | 07/23/58 | *to 1400 mi | 20 min |
| Squirrel monkey | Jupiter Rocket/US | 12/13/58 | *to 290 mi | |
| Rhesus monkey, squirrel monkey | Jupiter Rocket/US | 05/28/59 | *to 300 mi | |
| Black C57 mice | Discoverer III/US | 06/04/59 | * | |
| Black C57 mice (12) | Jupiter/US | 09/16/59 | * | |
| Rhesus monkey | Hermes 1/US | 12/04/59 | *to 280,000 ft | 13 min |
| Rhesus monkey | Hermes 2/US | 01/21/60 | *to 49,000 ft | 8 min |
| Black mice (21), white mice (19), rats (2) | Lambda Sputnik V | 08/19/60 | *to 450 km | |
| Black C57 mice | Atlas/US | 10/13/60 | *to 650 mi | |
| Chimpanzee | Mercury 2/US | 01/31/61 | *to 251 km | 18 min |
| Mice | 4th Spaceship/USSR | 03/09/61 | 183-249 mi | 1 hr 45 min |
| Chimpanzee | Mercury 5/US | 11/29/61 | 99-146 mi | 3 hr 20 min |
| White rat | Aerobee Rocket/US | 12/05/67 | * | 460 sec |
| <u>Macaca nemestrina</u> | Biosatellite III/US | 06/29/69 | orbital | 8.5 d |
| Pocket mice | Apollo 17/US | 12/05/72 | orbital | 12.0 d |
| Rats | Cosmos 605/USSR | 10/31/73 | orbital | 22.0 d |
| Rats | Cosmos 690/USSR | 10/22/74 | orbital | 22.0 d |
| Rats (25) | Cosmos 782/USSR | 11/25/75 | 226-405 km | 19.5 d |
| Rats, wistar (30) | Cosmos 936/USSR | 08/03/77 | 224-419 km | 18.5 d |
| Rats, wistar (37) | Cosmos 1129/USSR | 09/25/79 | 226-406 km | 18.5 d |
| <u>Macaca mulatta</u> (2), rats, wistar (10) | Cosmos 1514/USSR | 12/14/83 | 226-288 km | 5.0 d |
| Rats (6) | STS 8/US | 08/30/83 | orbital | 8.0 d |
| Rats (6) | STS 41-B(11)/US | 02/03/84 | orbital | 8.5 d |

TABLE 1.- CONCLUDED

| Species (N) | Mission/country | Date launch | Altitude | Duration |
|--|------------------|-------------|------------|----------|
| Squirrel monkeys (2), rats (24) | Spacelab 3/US | 04/29/85 | orbital | 7.5 d |
| Rhesus monkey | Cosmos 1667/USSR | 07/10/85 | 222-297 km | 7.0 d |
| Rats, wistar (10), rhesus monkeys (2) | Cosmos 1887/USSR | 09/29/87 | 224-406 km | 12.5 d |

†Compiled in part from the following:

Anderson, M.; Rummel, J. A.; Deutsch, S., eds.: BIOSPEX: Biological Space Experiments, A Compendium of Life Sciences Experiments Carried on U.S. Spacecraft. NASA TM-58217, 1979.

Gazenko, O. G.; and Ilyin, E. A.: Investigations on-board the biosatellite Cosmos 83, Adv. Space Res., vol. 4, no. 10, 1984, pp. 29-37.

Halstead, T. W.; and Dufour, P. A., eds.: Biological and Medical Experiments on the Space Shuttle 1981-1985, Life Sciences Division, Office of Space Science and Applications, NASA Headquarters, Washington, DC, 1986.

Simmonds, R. C.; and Bourne, G. H., eds.: The Use of Nonhuman Primates in Space, Proceedings of a symposium held at Ames Research Center, Moffett Field, California, December 2-4, 1974, NASA CP-005, 1977.

SUMMARY OF CONCLUSIONS REACHED IN WORKSHOP AND RECOMMENDATIONS FOR LIGHTING ANIMAL HOUSING MODULES USED IN MICROGRAVITY RELATED PROJECTS

Conclusions

This workshop consisted of formal 45-minute seminars by 14 speakers followed by informal group discussions. In the afternoon of the second day, all workshop participants (speakers and attendees) gathered for a round-table "executive session" in which specific questions were addressed, discussed, and final recommendations made. The recommendations listed below, therefore, represent a consensus based on data presented by the speakers and data offered by others during the executive session discussion. It should be noted that the recommendations are meant to provide a basis for valid and meaningful scientific animal investigations in space that can be correlated with the myriad ground based studies that currently exist and that are to be performed in the future. The recommendations were based on available information and may require modifications as future studies expand our understanding of this subject.

Recommendations

1. General

These lighting recommendations should be utilized for all space flights regardless of duration. The recommendations must apply to ground based studies to which flight data might be cross correlated.

2. Nonhuman Primates Versus Rodents

Separate standards should exist for nonhuman primates versus rodents. The standards should apply for all varieties within a grouping; i.e., the rodent standard should apply to pigmented (Long-Evans type) and albino (Sprague-Dawley type) rats; the nonhuman primate standards should include monkeys of the genera Macaca and Saimiri (e.g., rhesus and squirrel monkeys). The rodent standards are meant to include mice (Mus) and rat (Rattus) species. Different requirements might apply for other rodents or non-human primates particularly if their characteristic light-related behavior pattern is reversed from that of the species listed above (i.e., diurnal versus nocturnal).

Note: Considerable discussion centered on the question of albino versus pigmented animals (particularly in light of data provided by Williams). Two views were expressed.

- a. Because of the albino animal's "handicap"—inability to control the amount of light impinging on its retina—"what it gets is what it sees." Manipulation of environmental illumination under these circumstances, quite simply, constitutes an independent variable. The "normal" animal's capacity to modulate photic input through autonomic mechanisms has been eliminated through selective breeding.
- b. For the majority of experiments, "normal" pigmented animals would probably be preferred. They might reasonably be expected to be less vulnerable to transient, uncontrolled perturbations in the photic environment, and be functionally consistent (i.e., not variously damaged by prior exposure to light).

3. General Light Conditions

The light source should be diffuse and emanate from one surface, thus providing a directional cue. Point sources of light should be avoided. Light intensity should not vary more than 20% according to position within the cage (note that this is when food and other paraphernalia are in place). Light parameters (e.g., intensity) should not vary more than 15% over the duration of a flight. It is recommended that light intensity in each cage be measured before and after the flight to document changes that might occur due to such factors as bulb age or power drop-off (e.g., battery drain). If possible, light parameters should be measured routinely in flight to confirm compliance with the standards. The concept of feedback clamping the intensity was discussed as a possible method to limit variability over time. It should be noted that some animals (particularly rodents) modulate their photic input within a given microhabitat by seeking shelter away from the light source (see Lynch). A uniformly lit cage restricts this mechanism.

4. Light Spectral Qualities

The light source should produce a spectral profile that simulates as close as possible natural sunlight. Sunlight at 12:00 hr at a middle latitude on June 21 is to be the standard reference. Very complete solar global spectral irradiance data for Tanashi, Tokyo, Japan (35° 43' north latitude, 73 m above sea level) can be found in the following: Habu, M.; Suzuki, M.; and Nagasaki, T.: Measurement of the solar spectral irradiance at Tanashi, Tokyo (I, II, III). Researches of the Electrochemical Laboratory Nos. 812, 813, and 830; 1981, 1981, 1983. Excerpts of this data may be found in Thorington, L.: Spectral, Irradiance, and Temporal Aspects of Natural and Artificial Light. The Medical and Biological Effects of Light, Wurtman, R. J., Baum, M. J., and Potts, J. T., eds., Annals of the New York Academy of Sciences, vol. 453, 1985. UV-A and UV-B components should be present. Filters should be available to remove (block) these components if an investigator so desires. Because the spectral properties of most lamps currently available can change over time, it is recommended that the manufacturer specify a useful life (in hours) and that lamps be logged and monitored to stay within these limits during a particular mission. It is also recommended that lamps be turned on for a period of time prior to use in a mission to allow them to stabilize. The period of time ("burn-in time") should be recommended by the manufacturer.

5. Light Intensity

Considerable discussion ensued pertaining to light intensity. It was the consensus that, from an engineering standpoint, every effort should be made to achieve the nominal intensity. When this is not possible, only values within the acceptable range of intensity should be considered. Due to possible permanent retinal effects of bright light exposures to rodents (see Williams), these animals should be raised under the same lighting standards as proposed for space flights. At no time should animals be exposed to light intensities outside the "acceptable" range (also see recommendation 10 below). It was suggested that future investigators might desire greater light intensities for some applications. It is, therefore, recommended that lighting systems be engineered with the capability to produce up to 1000 lux (410 $\mu\text{W}/\text{cm}^2$, sunlight simulating light) intensity in rodent habitats and nonhuman primate habitats. If possible, the intensity should be continuously variable (infinite increments or analog).

a. Lights on phase

1) Rodents

The nominal light intensity at the position of the animal in mid cage should be 40 lux (16.4 $\mu\text{W}/\text{cm}^2$), unless otherwise specified by the investigator. If this is not possible, a light

intensity at the beginning of the flight within the range 5 to 75 lux (2.1-30.8 $\mu\text{W}/\text{cm}^2$) is acceptable. Once an intensity is selected, that intensity should be maintained constant, and not vary more than 15% over the duration of the flight (see recommendation 3). The light level must never be less than 5 lux (2.1 $\mu\text{W}/\text{cm}^2$) during the light phase.

2) Nonhuman primates

The nominal light intensity at the level of the animal's head should be 300 lux (123 $\mu\text{W}/\text{cm}^2$), unless otherwise specified by the investigator. If this is not possible, a light intensity at the beginning of the flight within the range 70 to 1000 lux (28.7-410 $\mu\text{W}/\text{cm}^2$) is acceptable. Once an intensity is selected, that intensity should be maintained constant, and not vary more than 15% over the duration of the flight (see recommendation 3). The light level should never be less than 70 lux (28.7 $\mu\text{W}/\text{cm}^2$) during the light phase.

It should be noted that there was considerable concern about the recommended low intensity value. Hoban and Fuller report that their animals show normal circadian entrainment at these levels. Brainard, however, expressed concern that the neuroendocrine system of nonhuman primates may require an intensity of 150 lux (61.5 $\mu\text{W}/\text{cm}^2$) or more of white light for normal function.

b. Lights off phase

The standard applies for both rodents and nonhuman primates. Light intensity during the "dark phase" should be less than 0.002 $\mu\text{W}/\text{cm}^2$. The spectrum of this light should be restricted to wavelengths greater than 640 nm.

6. Dark Phase Monitoring

It is recommended that infrared monitoring of the animals be possible during the dark phase (either direct type or infrared video monitoring). When using infrared monitoring, a long pass cutoff filter should be used in front of the infrared light source (e.g., 715 nm cutoff, less than 0.001% transmission below 660 nm). If infrared monitoring is not possible, and monitoring during the dark phase is required, the habitats should allow for direct visual monitoring during the dark phase. It was the consensus that dark phase monitoring and effects of dark phase light have not been adequately studied. It should be noted that very little literature could be found on the effects of low level lighting during the dark phase. Specific wavelengths and intensities appropriate for each species need to be determined. The relative intensity of the light phase versus the dark phase may be an important factor. Provisionally, for situations utilizing direct visual monitoring, the lighting during the dark phase should be constant using wavelengths at 640 nm and above (not to exceed 0.20 $\mu\text{W}/\text{cm}^2$) for observation of rodents and diurnal nonhuman primates. Note, if this observational ("red") light system is used for direct visual monitoring, then it should remain constantly on during both the light and dark phases of the light cycle (i.e., it should remain constantly on, independent of the "white" light on/off cycle). It was suggested that some investigators might require constant video monitoring (including constant monitoring during the dark phase).

7. Light Intensity Measurement

Light intensity should be reported simultaneously both in radiometric terms, irradiance (e.g., $\mu\text{W}/\text{cm}^2$ cumulative from 290-800 nm), and in photometric terms, illuminance (e.g., lux with exact light source, model and manufacturer and distance from light source specified). The spectral power distribution of the

light source should be measured or appropriately referenced. This recommendation stems from the fact that many published animal studies do not provide adequate information to accurately reproduce a given light environment. Because many labs do not have the capability to measure spectral power distribution, and since most of the earlier literature reports illuminance units, it was felt important to use both methods. An approximate conversion factor for light (290-770 nm) which simulates sunlight (CIE D-5500 K, CRI 91) is: $\text{lux} \times 0.41 = \mu\text{W}/\text{cm}^2$ of white light (Brainard).

8. Light Cycle Control (Light/Dark Timing)

Control for the light/dark cycle shall be variable. However, for standard experiments with rodents, the light cycle shall be 12 hr light/12 hr dark. For rodent species which have a strong seasonal biological rhythmicity (circannual physiology) a "long" photoperiod should be employed (e.g., 14 hr light/10 hr dark). For standard experiments with squirrel monkeys, the light cycle shall be 12 hr light/12 hr dark. For standard experiments with rhesus monkeys, the light cycle shall be 16 hr light/8 hr dark. The system should allow for manual override (i.e., allow lights to be turned on or off). It was agreed that many non-standard lighting scenarios might be required. Accordingly, the timing of light and dark should be programmable in 1 min or less increments and accurate to within plus or minus 1 min. The number of hours in one light/dark cycle may be other than 24 hr, and the length of the lights on phase should be variable in 1 min increments to a condition in which the lights are constantly on. The timing mechanisms should allow for this contingency. It should also be noted that some experiments may require constant light or constant dark exposure for the full duration of the flight.

9. Avoidance of Viewing or Light Source Blocking Problems

Because animal excrement, dander, or feed/water particles may be potential problems in blocking light sources, viewing, and/or video monitoring ports, provisions should be made to keep these clear or regularly cleaned. If a continuous plastic-type film system is used, it should not appreciably reduce the intensity (block) or alter the spectrum of the light source by "filtering."

10. Importance of Strict Lighting Parameters Control

It was the consensus that lighting parameters be strictly maintained. Of particular concern was inadvertent light exposure during the animal's dark phase. Since even very short duration light "flashes" might be disruptive to the biological timing system, it is recommended that all inadvertent light exposures be avoided and that every effort be made to "light proof" the animal cages to prevent outside light from "leaking" into the cages. It was suggested that these light exposures be avoided when personnel are routinely observing or maintaining the animals (including transfer between cages). It should be noted that inadvertent bright light exposure, even during the animal's "lights on" phase, may be damaging (see Williams).

11. Light/Dark Cycle Record

It is recommended that the animal habitat equipment include the capability of continuously recording actual timing of periods of light exposure and periods of darkness over the entire duration of the mission (i.e., actual times of lights on and lights off, as well as inadvertent exposures). It is preferred that this record be generated directly from a light sensing transducer within the cage and not indirectly (e.g., from the light power circuits).

ANIMAL HABITATS: Scientific Requirements, Engineering Limitations

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The objectives of this presentation are to describe animal flight hardware that has been used or is being developed by the Space Life Sciences Payloads Office of the NASA Ames Research Center and to point out the various aspects of flight experiment hardware development.

As Dr. Holley pointed out in his introduction, the initial reason for this conference was a concern about the lighting levels in the animal habitat which is planned to fly in 1990 on the SLS-1 (Space Life Sciences One) Spacelab. This habitat is called the Research Animal Holding Facility or RAHF. It became evident, however, that we should also delineate the lighting requirements for the other habitats now in development. These are listed in Table 1 along with a brief characterizations of each habitat. Briefly, they are the RAHF, the Animal Enclosure Module (AEM), the satellite called Lifesat, and the Modular Habitat to be used on Space Station. These will be described in some detail.

In Figure 1, two configurations of the RAHF are shown. The Rodent RAHF can hold 24 rats in 12 double cages; the Primate RAHF can hold four squirrel monkeys in four individual cages. These two RAHFs, which were conceived in the early 1970's, flew on the Spacelab 3 flight (SL-3) in early 1985 with 24 rats in the Rodent RAHF and two squirrel monkeys in the Primate RAHF. From this flight it was determined that certain modifications to the RAHF and the individual cages should be made. These new modifications are indicated in Figure 1.

The RAHF, of course, has all the components for the proper maintenance of rats or squirrel monkeys in space. These include a cage with feeding and watering capability, a waste tray to capture and contain debris, and an environmental control system (ECS) to control gas composition, temperature, humidity, air flow to entrain the debris, and a lighting system. The RAHF was designed for 7-10 day flights.

Figure 2 shows the new rodent cage that is now in development. With regards to lighting, it is basically the same as the SL-3 cage with a major difference being the addition of a 150 micron screen on the top in addition to the 1/4" grid to hold the rats in the cage. This screen decreases the light levels but is necessary to retain particulates within the cage.

The primate cage used on SL-3 was an open cage with a feeder and watering system. As with the rodent cages the lights were positioned opposite the surface normally considered the floor, i.e. opposite the waste tray.

On display, are both an original SL-3 rodent cage and the new design SLS-1 cage. Also available is a light bar with four 24 EX GE light bulbs and a 2 volt power supply. This light bar can be placed over each cage to give one a true feeling of the light levels and quality actually seen by the rats in either cage.

People at this workshop who have been involved with the design and development of the RAHF are Gary Bowman and Dale Buckendahl. They can clarify any questions that might arise.

Another unit which is to fly on SLS-1 is the Animal Enclosure Module or AEM shown in Figure 3. It's a portable design which just fits into a shuttle mid-deck locker. If you have seen photos of this part of the shuttle orbitor, there are a number of lockers on the wall where the crew can stow equipment, food or whatever. These lockers are approximately 10"x17" by 20" deep. A prototype AEM is on display. It is a complete functioning unit with lights, fans, and a filtering system. However since its use is not strictly controlled, and since it is used for various tests which may damage it or decrease its useful life, it will not qualify for flight.

The AEM gang cages 5 or 6 rats depending on their size or on other circumstances. Of course, for certain studies it is appropriate, even necessary to cage the experimental rat individually as with the RAHF. However, for many experiments, gang caging is a perfectly acceptable means of housing. In fact, in some cases, it's preferable.

The original AEM was designed for experiment which were proposed by high school students as part of the National Science Teachers - NASA Student Involvement Project. The basic student AEM has been taken and improved upon. For example, when it flew on STS-11 in 1984, potatoes were used as a source of water. Probably 9 potatoes to 6 rats were used. They were intermingled and the rats could be seen crawling among the potatoes during flight. They seemed perfectly happy. However, the water supply was obviously uncontrolled. A canister containing water is now being developed. It has 4 Lixits (water outlets) on it. It holds almost 2 liters of water and can maintain 5 rats for 10 1/2 days. The water amount

may not be sufficient for 6 rats but the AEM is, indeed, capable of housing them as has already been demonstrated on the STS-11 flight.

The Lifesat (Life Sciences Satellite) is shown in Figure 4. This is to be an unmanned free-flier of the old style. It is basically a recoverable rocket and is now in the design phase. The Lifesat program study team includes Pearl Cheng and John Givens. Workshop attendees involved in the Lifesat are Jack Dyer and Dick Schaupp - Engineering; and Dr. Lisabeth Kraft - Veterinary Science. The Lifesat is being sized for 12 rats for 24 days. It could fly longer with other organisms or with plants. When it is given the "Go ahead" it should be ready to fly in 3 1/2 years and then should be able to fly a new payload every four months thereafter.

Illustrated in Figure 5 is a cross sectional diagram of the rat capsule. The cage height is 7 inches and the floor space is 60 square inches per animal. Main features are the fans for air flow and debris movement, back flow shutters to contain the feces in the feces trap, heat exchangers, and a chemical bed for CO₂ odor control. Not shown are the O₂ and N₂ bottles which are outside the animal unit itself.

Figure 6 is a top view of the Lifesat rat capsule. The food bars will be wired to the side. As yet, it has not been decided as to where the lights will be placed or their type. However, the results of this workshop will be an important factor. The water Lixits are grouped as shown and it is anticipated that a video camera might occupy alternate positions with the Lixits. Refer again to Figure 4, the reentry vehicle. The payload containing the rats is within the forebody. It will be 64 inches in diameter and about 76 inches long. The animal module within will be 42 inches in diameter and 24 inches high. On top there will be a small cylinder 20 inches in diameter by 11 inches high containing portions of the environmental control system.

The Space Station Modular Habitat illustrated in the next series of diagrams is intended to be the final word on animal habitat systems for the space program. It will be used for rats, mice, squirrel monkeys, and Rhesus monkeys and for other small animals which will be exposed to extended periods in space in excess of 90 days. Plans are to have the systems automated such that the animals can be unattended for 90 day periods.

Workshop attendees involved in the design of the Space Station Habitat are Jenny Kishiyama - Engineering Management, Linda Swan - Engineering, Dr. W.E. Hinds - Science, Dr. Lisabeth Kraft - Veterinary Science.

The Space Station Modular Habitat is presently in its design phase. The design group will also make use of the recommendations that come from this workshop regarding intensity, type, and positioning of the lighting.

The first diagram in this series, Figure 7, is an overview of the Space Station unit housing the animal Modular Habitats. The centrifuge is located in the back of the station. A number of habitat units are located on the periphery of the centrifuge. These units can be interchanged with habitat units in the racks. Also shown is a work station wherein modular units can be placed for manipulation or treatment of the animals.

The next diagram, Figure 8, shows a standard double rack with 6 modular units in it. Three of these, as indicated, are for rats. The size of the modular unit will be 16 inches square by about 22 inches deep. They will be removable and portable. Figure 9 shows the Modular Habitat Operating Concept. The Modular Habitats, of course, could be used for the ground controls and during the initial testing. A modular habitat could also be taken up into orbit in the mid-deck since it is self-contained. It is not necessary to have an entire Spacelab. The Habitat Concept allows for incremental expansion. The individual units could contain rats, mice or squirrel monkeys.

More detail on the RAHF and the AEM will be presented to the workshop since these units have lighting concerns which require an early resolution. The first flight of the RAHF, the only flight so far, was on SL-3. This was launched April 29, 1985 and was recovered seven and a half days later on May 6, 1985. On SL-3 the Rodent RAHF had 24 rats. The Primate RAHF had two squirrel monkeys. It was capable of carrying four monkeys but only two could be found that met the stringent requirements which had been established. The flight configuration is illustrated in Figure 10. The RAHF, as previously stated, was designed essentially for 7 day flights, although it could go for 10 1/2 days. It was the first flight of the RAHF, so SL-3 was primarily an engineering test flight. Scientifically, it was a highly successful flight also.

The rats on SL-3 were pathogen-free rats. There were 12 large rats averaging 350 grams and there were 12 small rats of about 200 grams. Some were instrumented with BTS (biotelemetry systems) to measure deep body temperature and heart rate. The two monkeys were uninstrumented. The temperature was 23°C for the rats. The temperature for the monkeys was about 27°C. Humidity was on the order of 30% to

70%. There was a forced air flow. The CO₂ was less than 1% and the oxygen partial pressure was normal. It was basically a one atmosphere environment. The lighting was 12/12 light/dark and the intensity was about 70 lux in the rat cage, although that was an average figure. The bulbs used were 24 EX from GE, which have a color temperature of about 2800°. Much of the basic work that went into designing the RAHF started very early in the 70's. Some of the work on lighting that was done during the period was with Franz Halberg who was a Principal Investigator at that time. Just about every aspect of the animal habitat was investigated in excruciating detail. So, there was never any concern about the scientific adequacy of the SL-3 Habitat. Nor has there ever been any fears or concerns about the light levels since that was looked into, albeit years ago. But certainly it was looked into in detail. And as previously stated, extensive preflight studies indicated there was an essentially normal response by the animals to the RAHF in its final configuration. In the flight rats, all the changes seen could be reasonably attributable to the weightless state. This conclusion is based on a comparison to the extensive ground controls that were run at that time.

Figure 10 shows an overview of what the SL-3 Life Sciences Payload looked like. On the right is the monkey RAHF and the rodent RAHF is on the left. The mirror is part of the camera system and allows one to photograph the rats in the two lower cages. The lights for illuminating the cages were positioned just above the top lid which in this diagram would be on the right side of the cage. Each individual rat cage has 2 of the 24 EX GE bulbs. The monkey cage had 12 bulbs. These were positioned about 1/2" above the cage top in both cages. The mesh of the cage top was 1/4". The lights in both the SL-3 rodent cage and in the new SLS-1 rodent cage will be shown to the workshop attendees as a demonstration.

The top lid of the SL-3 cage was simply a 1/4" square grid made of 1/16" stainless wire. This resulted in a lighting level of about 70 lux on average. The SLS-1 cage top has an additional 150 micron screen over the grid. This cuts down the light to an average of about 42 lux. Next, Figure 11 shows a comparison of the lighting of these two cage types.

Figure 1, already discussed, also demonstrates some of the modifications that were made on the new SLS-1 primate and rodent RAHF and cages. In addition to sealing the cage for particles to the level of 150 microns, a powerful fan was put into the bottom of the RAHF. This is turned on whenever the cage's outer door is open or the cage is removed for servicing. The additional inward airflow insures that particulates or aerols will not escape into the Spacelab.

Spaceflight with animals as test subjects has been going on now for about 39 years. Many of these early flights were simply ballistic in nature and did not achieve orbit. They simply went up and down and usually impacted on landing and that was the end of the animal. But even though animals have flown in space these many years, the flights are few and far between and it's no simple matter to fly even the simplest space vehicle.

Preparation for space flight involving animals is a tedious and long-drawn out process which involves many people and at least 3 NASA centers. The process is even more complicated when a crew is on board the vehicle. In other words, if you have a manned spacecraft with animals on it, then it just becomes extremely complicated, and requires involved testing and documentation.

One important document that must be supplied to the operations people is the Science Requirements Document. The Science Requirements Document specifically details the animal requirements with regard to any and all the parameters which might have an effect on the scientific studies to be performed (see Table 2). These have been mentioned to some extent (see Table 4). The complexity of problems associated with flying animals in space is amply illustrated by this list of critical parameters that have to be followed.

For example, it will be important to control the temperature within the habitat. This could be a problem on a relatively simple spacecraft like the Lifesat. In that case, control of the temperature range desired is established prior to flight. The vehicle is selectively painted light and dark to help maintain the temperature by heat absorption/reflection. It is not an active control in the normal sense. Other things one would have to examine in close detail are of course the limits of the O₂ and the CO₂, and how fast the air flow should be, how many exchanges per unit time. Obviously the food and water delivery, the diet, and contaminant levels, characteristics of the animals, all have to be delineated. In other words, are they little animals, big animals, instrumented animals, rats, mice, female, male; and of course the lighting parameters?

The science requirements must be compatible with the physical capabilities of the vehicle. Simply requesting certain experimental conditions does not mean these are attainable, no matter what the scientific justifications are. Furthermore, the requirements have to be compatible with other elements of the payload.

Science requirements which are outside the normal capability of the vehicle or which could perturb other functions on the space craft require an adequate justification. For example, requesting 50 lux when 40 lux is ordinarily supplied would require documented scientific justifications since the Flight Project Managers have to integrate a whole complex of experiments and requirements, not just Life Sciences experiments. And above all, they have to be consistent with the budgetary capabilities of the performing organization. NASA, like any other Federal agency, has only a given amount of money budgeted for its various activities. For that reason, NASA must be judicious on how the money is used.

Also, because of the great time and expense involved, animals have to be shared. For example, on SLS-1, each rat will be parted out to 7 investigators. In other words, there are 7 experiments done on each rat. On the SL-3 flight there were much, much more than that. And in fact, there are going to be much more than that on SLS-1. But the 7 scientists who are the Principal Investigators have priority. Anything else that is done in addition cannot interfere with their experimental requirements. Nevertheless, it just adds a whole level of complexity in developing the science requirements. So, if more light is, indeed, needed, bigger light bulbs may be used. If incandescent bulbs are used, more heat will be generated and somebody is going to complain about the heat because they're doing a thermal regulation study. And so it goes on and on like this. Part of the job of the Payload Scientist is to try to mesh these various requirements and conflicts.

The other complicating factor is that on a manned space vehicle, NASA is extremely safety conscious. At the development level, excruciating exercises and justifications in terms of safety and reliability are required for everything that is being proposed. It's no simple matter; each item has to go through a materials approval cycle (Table 3). And just as another example, let us consider light bulbs again. They contain glass and if by chance they broke, they would create a safety problem. So they have to have a secondary containment in case the bulb is broken in flight. Then we need to make sure they work. They are pre-tested. The bulbs are run through a vibration and shock cycle test for reliability before they can be plugged into the unit and flown. A materials usage agreement based on these studies has to be developed for the bulbs. And this has to be processed and approved through the Mission Management Office and through the Spacelab Management Office or the Space Transportation System Office. So, it's not a simple act of changing a bulb.

Fluorescent tubes have been used aboard the shuttle and this will continue. However, they do require special engineering. This process will be discussed by others at this meeting. Suffice it to say here that fluorescent tubes do require alternating current and with their modifications they are expensive, costing around \$900 apiece. So one really has to consider carefully whether to use them or not.

Concerning light in animal space habitats, there are a number of questions for the workshop to address with regard to the RAHF and AEM. Questions are as follows:

- What is the light envelope with respect to intensity, periodicity, and signature (spectral qualities) that is acceptable for the majority of biological experiments with rats and subhuman primates in space flight where the scientific objectives are not primarily a study of the light environment itself?
- Is the RAHF lighting as presently configured adequate?
- Is the AEM lighting adequate?

The basic categories of experiments on SLS-1 will involve the skeletal, cardiovascular, muscle, and vestibular systems as well as hematology and immunology studies. Future flights, of course, will include a wider variety of experiments, including renal, endocrine, radiation, psycho-social behavior, animal development, genetics and aging as well as pulmonary physiology. One variable that we have is the type of animal (see Table 4). There may or may not be some distinction between albino and pigmented rats and mice with regard to light effects. Squirrel monkeys and Rhesus monkeys will also be used in space flight experiments. The other variables are the length of flight. The RAHF will fly 7-10 days, the Lifesat, 24 days, and the Space Station 90 days or longer. The light specifications will change with the length of the flight. There are also other considerations, as I have already mentioned, the light source, i.e. incandescent bulbs versus fluorescent bulbs. The initial cost must be considered. Should we use a bulb or a tube? How long will it last? Does it require AC or DC? How much heat does it generate? These are all factors that have some impact on the engineering. And, of course, it's the engineering complexity and safety considerations that are limiting factors.

As an example of the problems generated by engineering changes, we have the following comparison. Figure 11 is a comparison of the light levels

between an SL-3 cage which averaged about 70 lux and an SLS-1 cage which averages about 43 lux.

The same light source was used; everything was the same. The difference is the 150 micron screen on the top of the SLS-1 cage. Light levels in the AEM are shown in Figure 12. A grid of random points throughout the AEM was used to obtain an average value. Using 313 bulbs, on the average, about 18 lux are obtained, with the food bars in place. Light bulbs are less when no food bars are present. The value with food bars would be applicable since obviously there is going to be food bars initially. Also, it appears that there will be plenty of food left at the end of the flight. The 1820 bulbs, which are smaller, give an average of 14 lux. For comparative purposes, the mid-deck light level is stated to be 32 foot candles (344 lux) three feet above the floor. The operating temperature of the AEM with the 313 lightbulbs and fans on is about 77°F.

There is one final issue and this concerns the definition of the Visible Spectrum (Light). It is usually defined for human values in the literature as follows:

- | | |
|------------------------------------|----------------------|
| - Best and Taylor 1973 | About 370 to 740 n M |
| - IES Lighting Handbook 1981 (2-1) | 380 to 770 n M |
| - IES Lighting Handbook 1981 (3-3) | 380 to 760 n M |
| - Thorington - Anyas (1985) | 360 to 830 n M |

This comparison demonstrates part of the problem. As indicated, various sources give different values for the visible spectrum. This can be very confusing to the engineer who has to be very judicious in designing the lighting for a spacecraft. Likewise, what is the relationship between lux and microwatts/square centimeter? What should be used? We have to start off with definitions and fairly precise definitions at that. But certainly, we need definitions that are practical and applicable.

TABLE 1

ANIMAL HABITATS FOR SPACE FLIGHT
OF INTEREST TO THE WORK SHOP

- o RAHF (RESEARCH ANIMAL HOLDING FACILITY)
 - RAT UNIT - HOLDS 24 RATS IN 12 DOUBLE CAGES
 - SQUIRREL MONKEY UNIT - HOLDS 4 MONKEYS
 - UNRESTRAINED
 - RESTRAINED
 - FEED AND WATER ALIQUOTS (COUNTED), ENVIRONMENT CONTROL SYSTEM
 - DESIGNED FOR 7 TO 10 DAY FLIGHTS

- o AEM (ANIMAL ENCLOSURE MODULE)
 - PORTABLE UNIT DESIGNED FOR MID DECK LOCKER, NEEDS POWER INPUT
 - ORIGINALLY INTENDED FOR STUDENT EXPERIMENTS
 - FLOWN TWICE

- o LIFESAT
 - FREE-FLYING SELF-CONTAINED RECOVERABLE SATELLITE
 - 12 RATS FOR 24 DAYS, UNMANNED

- o SPACE STATION MODULAR HABITAT
 - RATS AND MONKEYS MAINTAINED FOR EXTENDED PERIODS I.E. 90 DAYS OF LONGER
 - ONLY OCCASIONAL SERVICING BY THE CREW

TABLE 2

SCIENCE REQUIREMENTS DOCUMENT

- o A SCIENCE REQUIREMENTS DOCUMENT SPECIFICALLY DETAILS ANIMAL REQUIREMENTS WITH REGARD TO ANY AND ALL PARAMETERS WHICH MIGHT HAVE AN EFFECT ON THE SCIENTIFIC STUDIES TO BE PERFORMED.

- o THAT WOULD INCLUDE:
 - HABITAT SIZE AND CHARACTERISTICS
 - OPERATING TEMPERATURE AND HUMIDITY
 - GASEOUS COMPOSITION AND LIMITS, O₂, CO₂
 - AIR FLOW
 - FOOD AND WATER DELIVERY
 - DIET
 - CONTAMINANT LEVELS
 - ANIMAL CHARACTERISTICS
 - LIGHTING PARAMETERS

TABLE 3

MATERIALS APPROVAL CYCLE
FOR FLIGHT QUALIFICATION
ON MANNED SPACECRAFT

- o LIGHT BULBS CONTAIN GLASS REQUIRING SECONDARY CONTAINMENT IN CASE OF FRACTURE
- o EVERY BULB MUST BE TESTED FOR VIBRATION/SHOCK AND RELIABILITY
- o MATERIALS USAGE AGREEMENT BASED ON TESTS MUST BE PROCESSED THROUGH THE MISSION MANAGEMENT OFFICE AND SPACELAB MANAGEMENT OFFICE. NORMALLY TAKES SEVERAL MONTHS.
- o CHANGING THE BULB TYPE REQUIRES A REPEAT OF THIS PROCEDURE.
- o FLUORESCENT TUBES ARE FLOWN ABOARD SHUTTLE AND SPACELAB BUT REQUIRE SPECIAL ENGINEERING TO CONTAIN AND RETAIN MERCURY AS WELL AS THE GLASS.
 - REQUIRES AC 400 Hz
 - COSTS \$900 EACH

TABLE 4

VARIABLES ON CONCERN

- o VARIABLES
 - TYPE OF ANIMAL
 - ALBINO RATS. PIGMENTED RATS, MICE
 - SQUIRREL MONKEY, RHESUS

 - LENGTH OF FLIGHT
 - 7-10 DAYS-RAHF AND AEM (RAT, SQUIRREL MONKEYS)
 - 24 DAYS-LIFESAT (RATS, MICE?)
 - 90 DAYS OR LONGER-SPACE STATION (RATS, MONKEYS, SQUIRREL MONKEYS AND RHESUS)

- o OTHER CONSIDERATIONS
 - LIGHT SOURCE
 - INITIAL COST, BULB OR TUBE LONGEVITY, POWER REQ. AC, DC, HEAT GENERATION, ENGINEERING COMPLEXITY, SAFETY CONSIDERATIONS

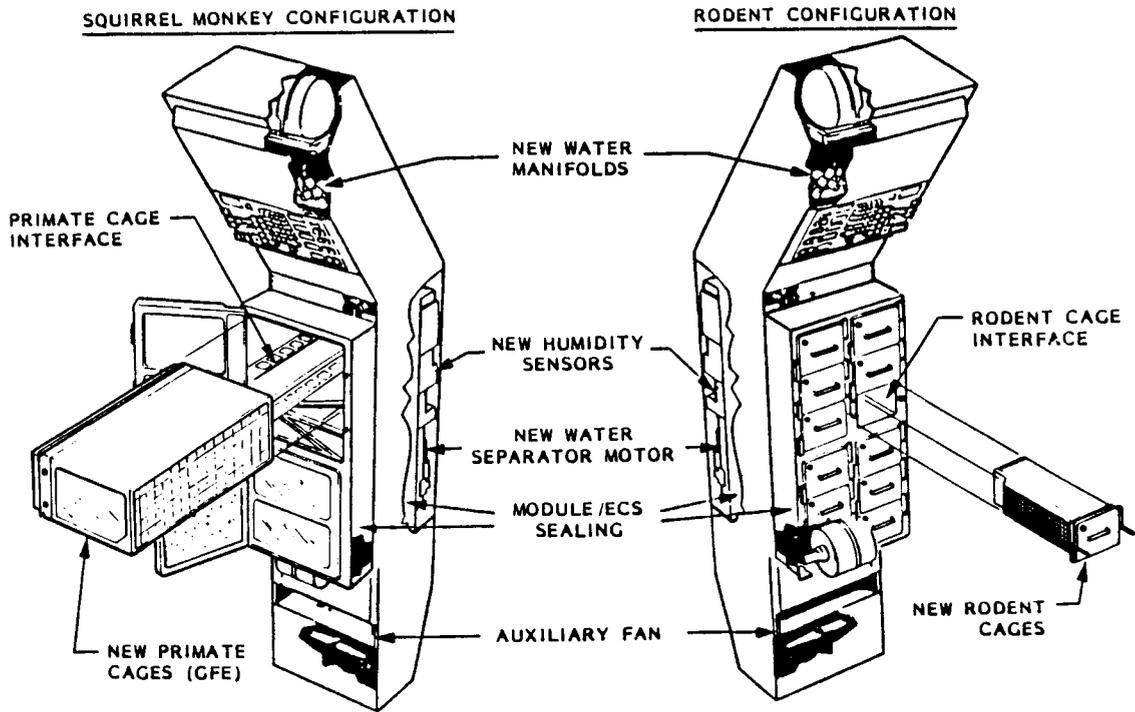
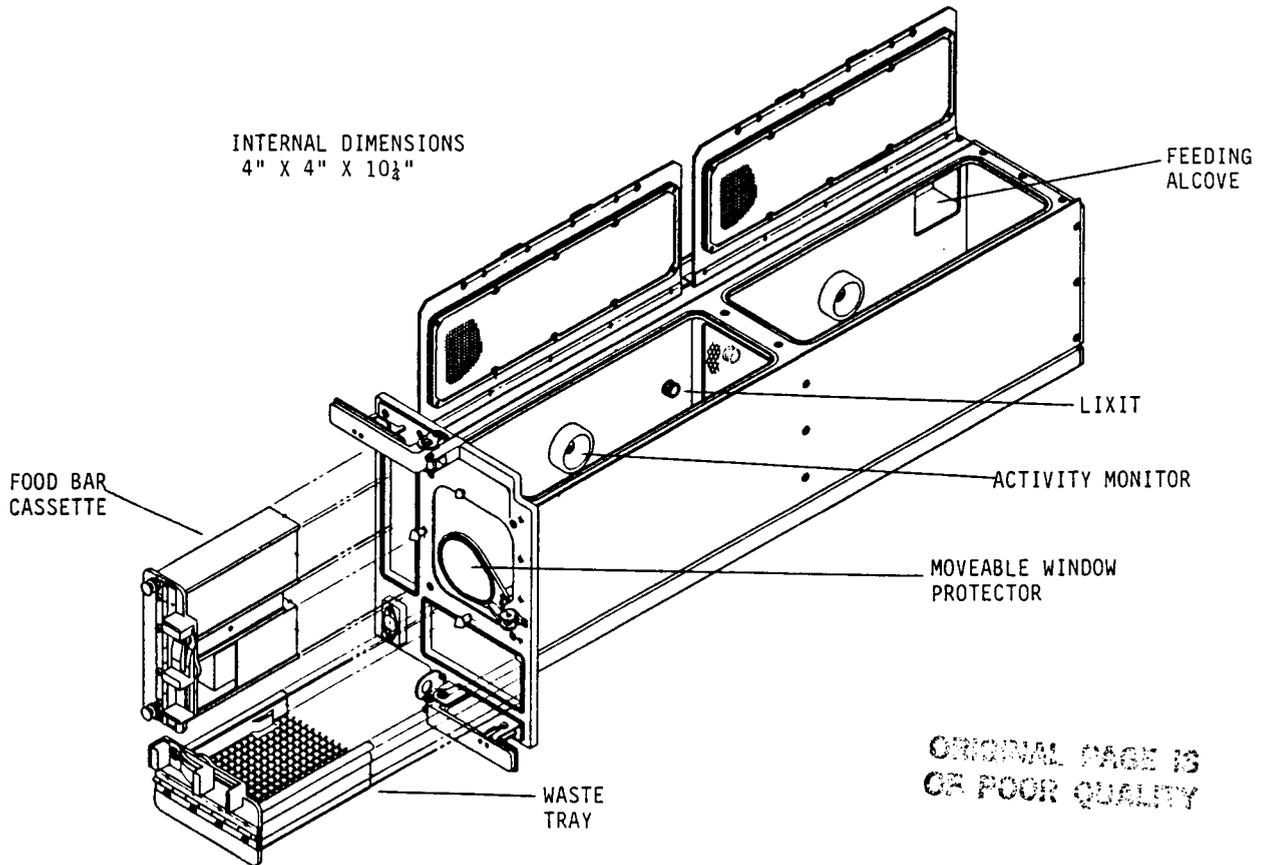


Fig. 1 RAHF post Spacelab 3 changes.



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Fig. 2 RAHF isometric view of cage.

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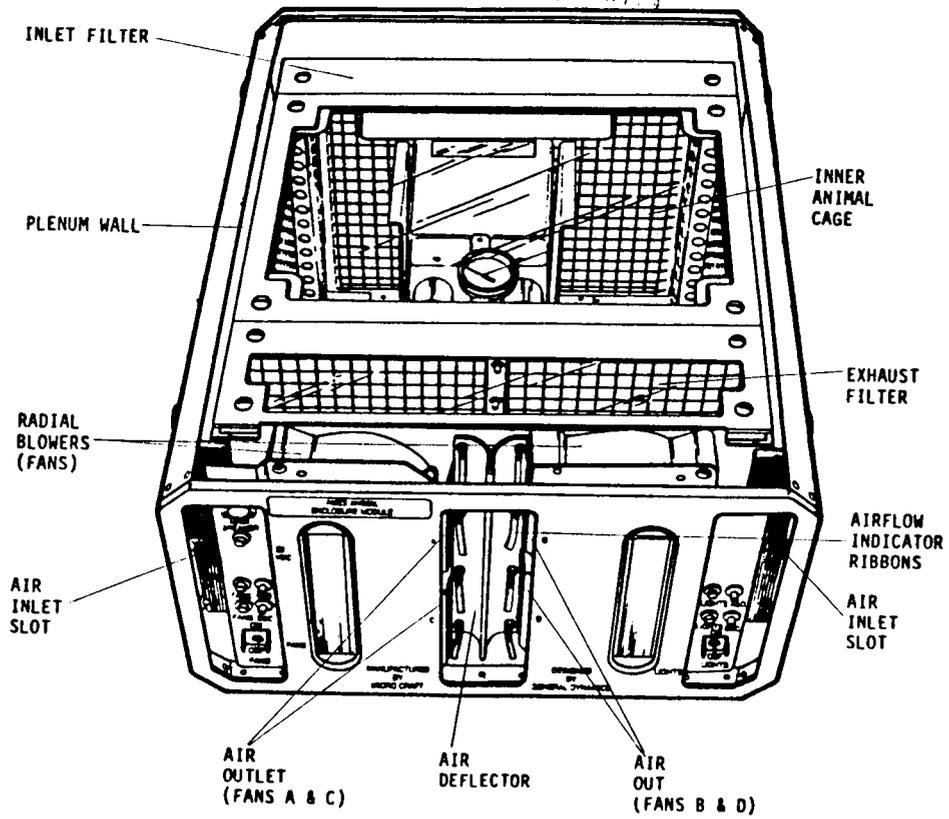


Fig. 3 Animal enclosure module.

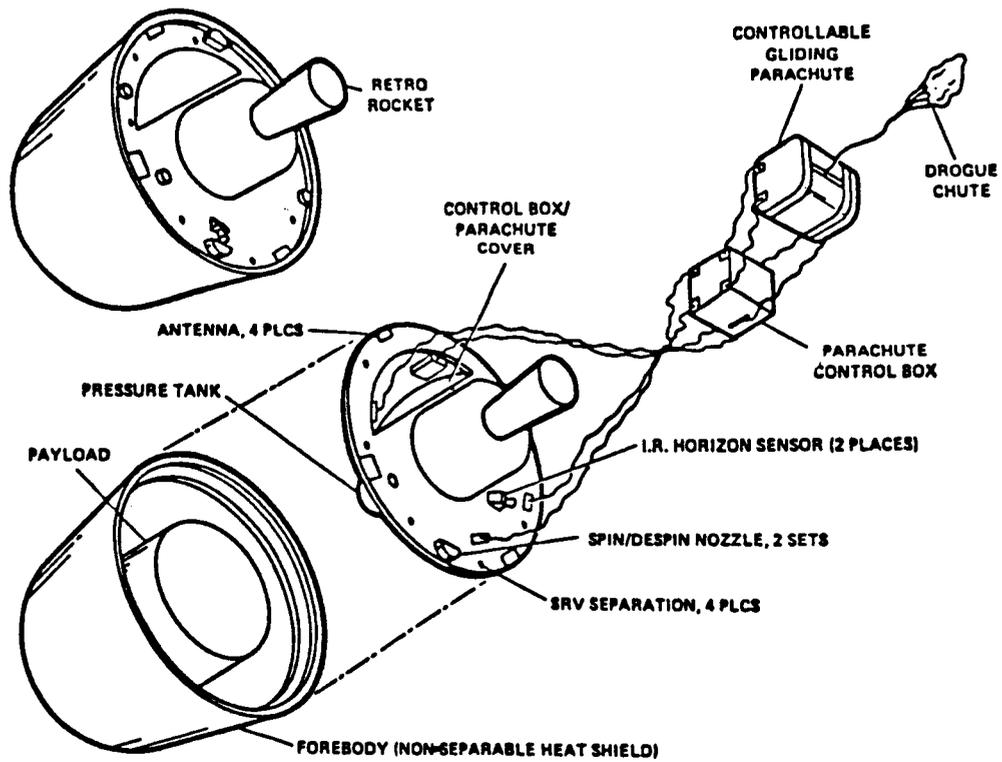


Fig. 4 RRS spacecraft concept.

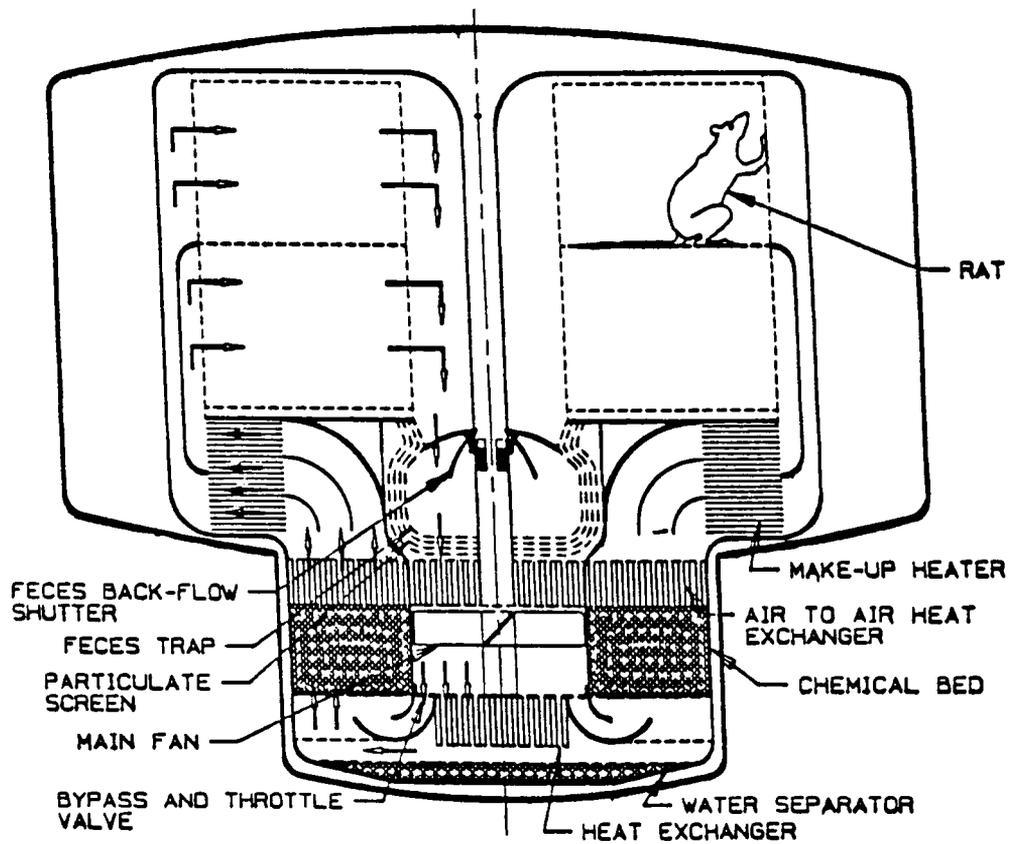


Fig. 5 Lifesat rat capsule cross section.

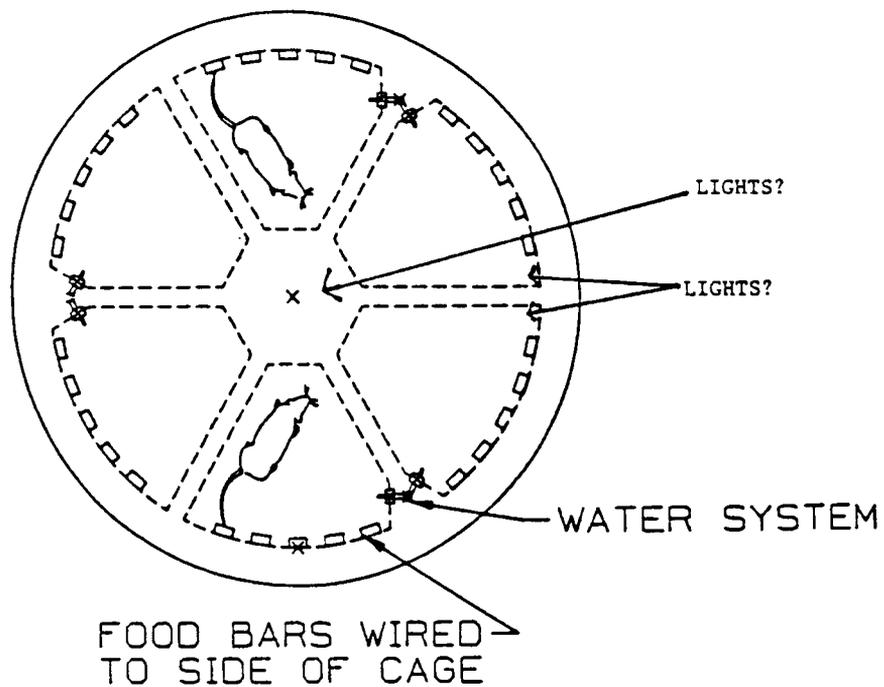


Fig. 6 Typical cage layout.

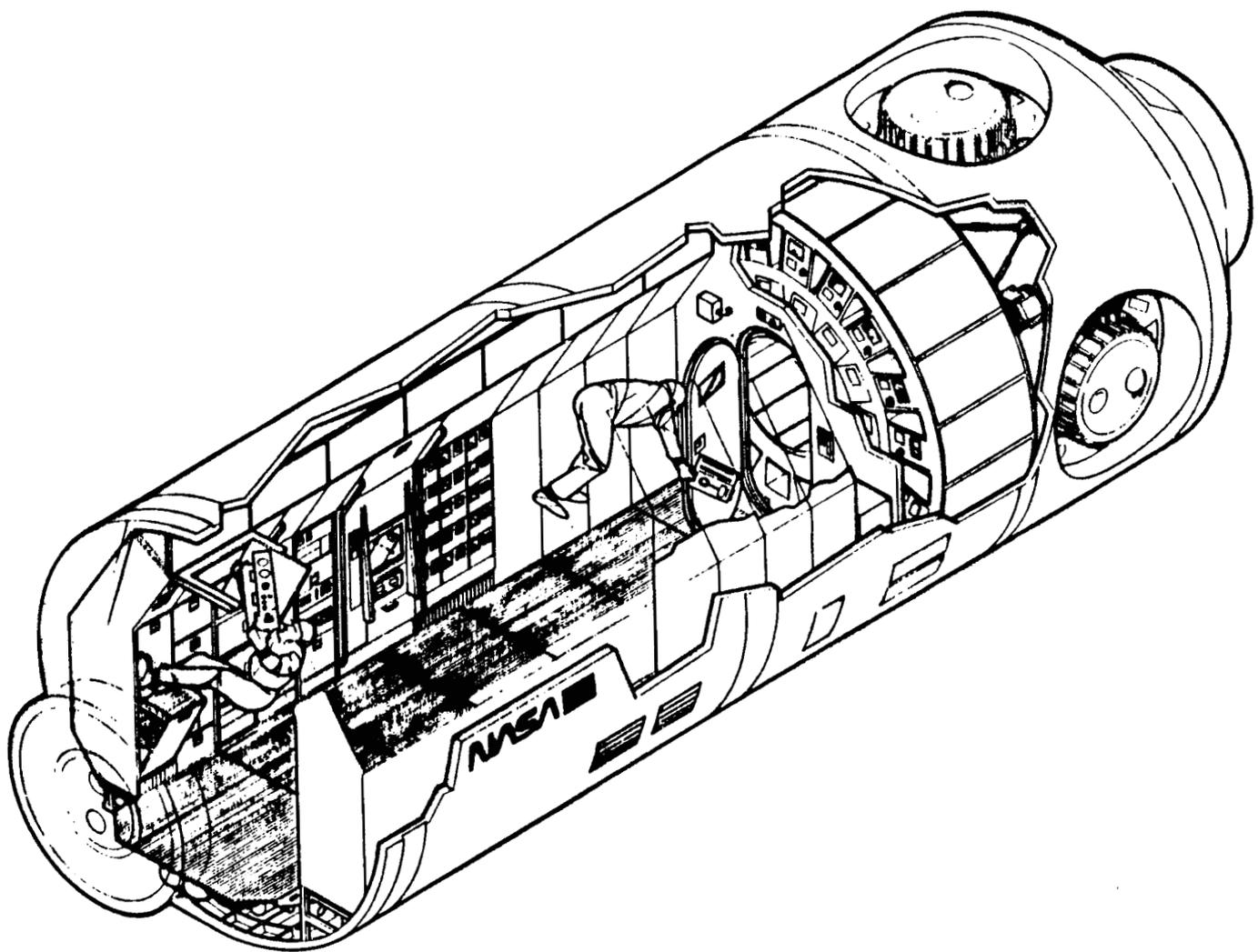


Fig. 7 Space Station unit with modular habitats.

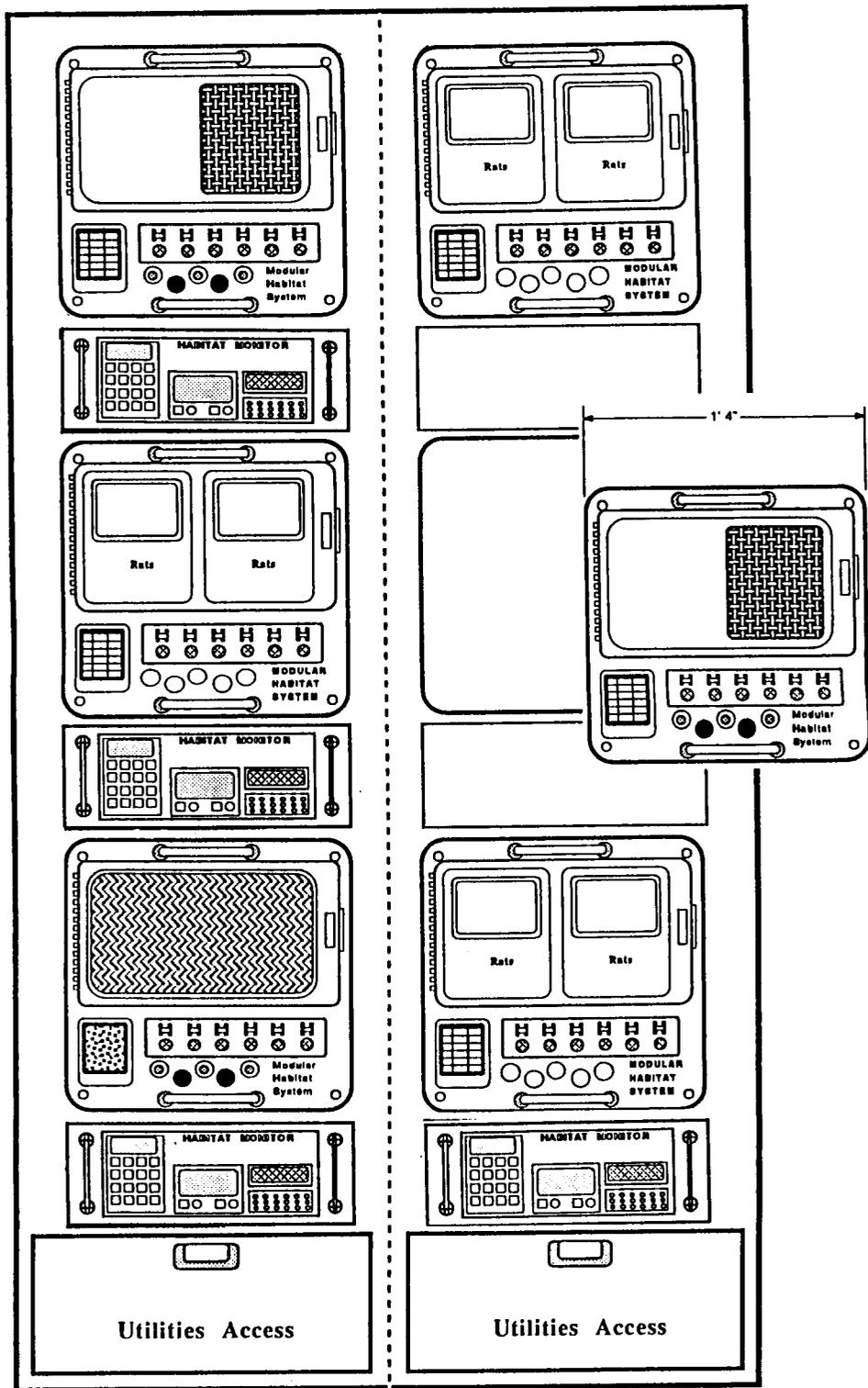


Fig. 8 Space Station life sciences: 80 in. x 42 in. double rack with modular habitats.

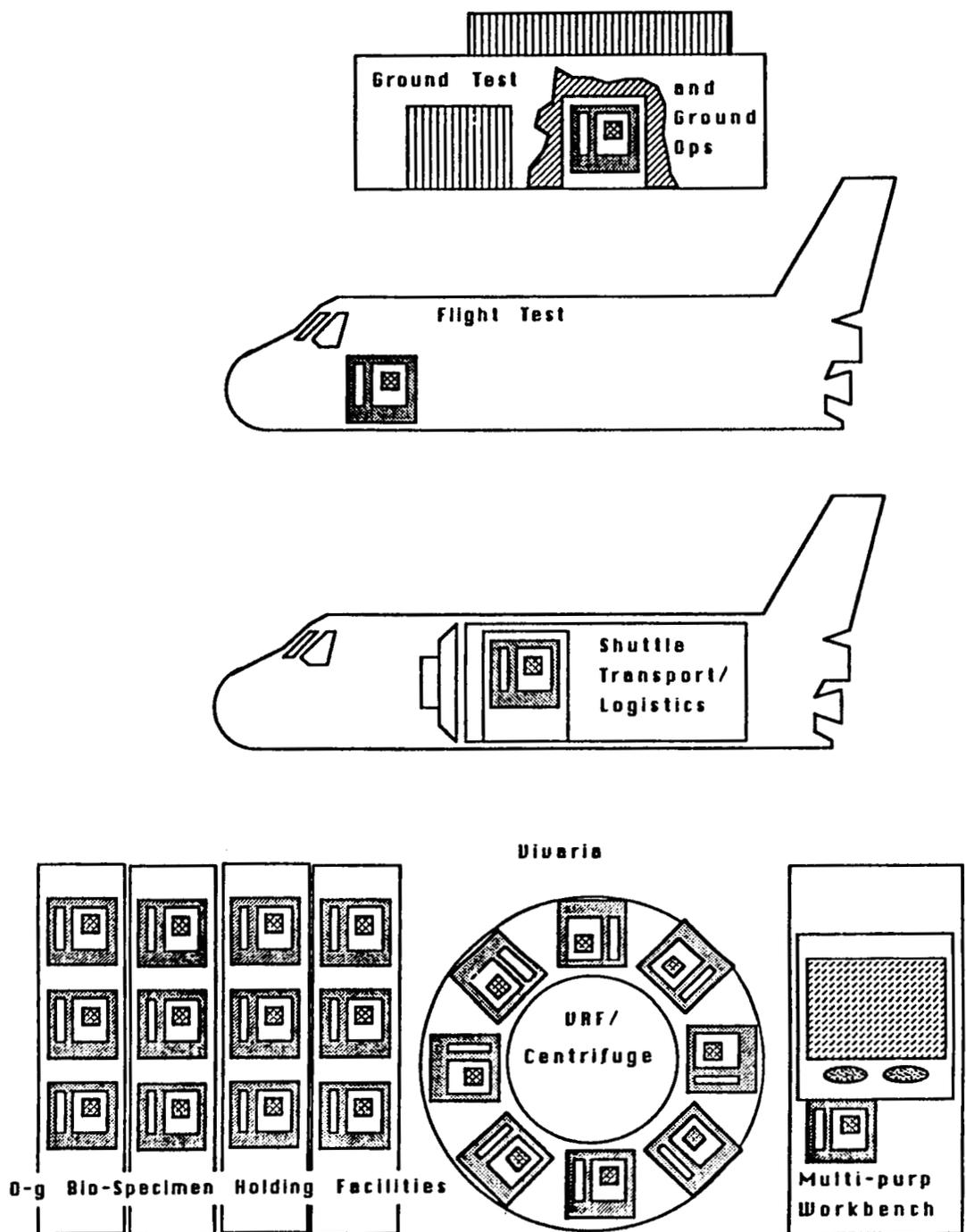


Fig. 9 Modular habitat operating concept.

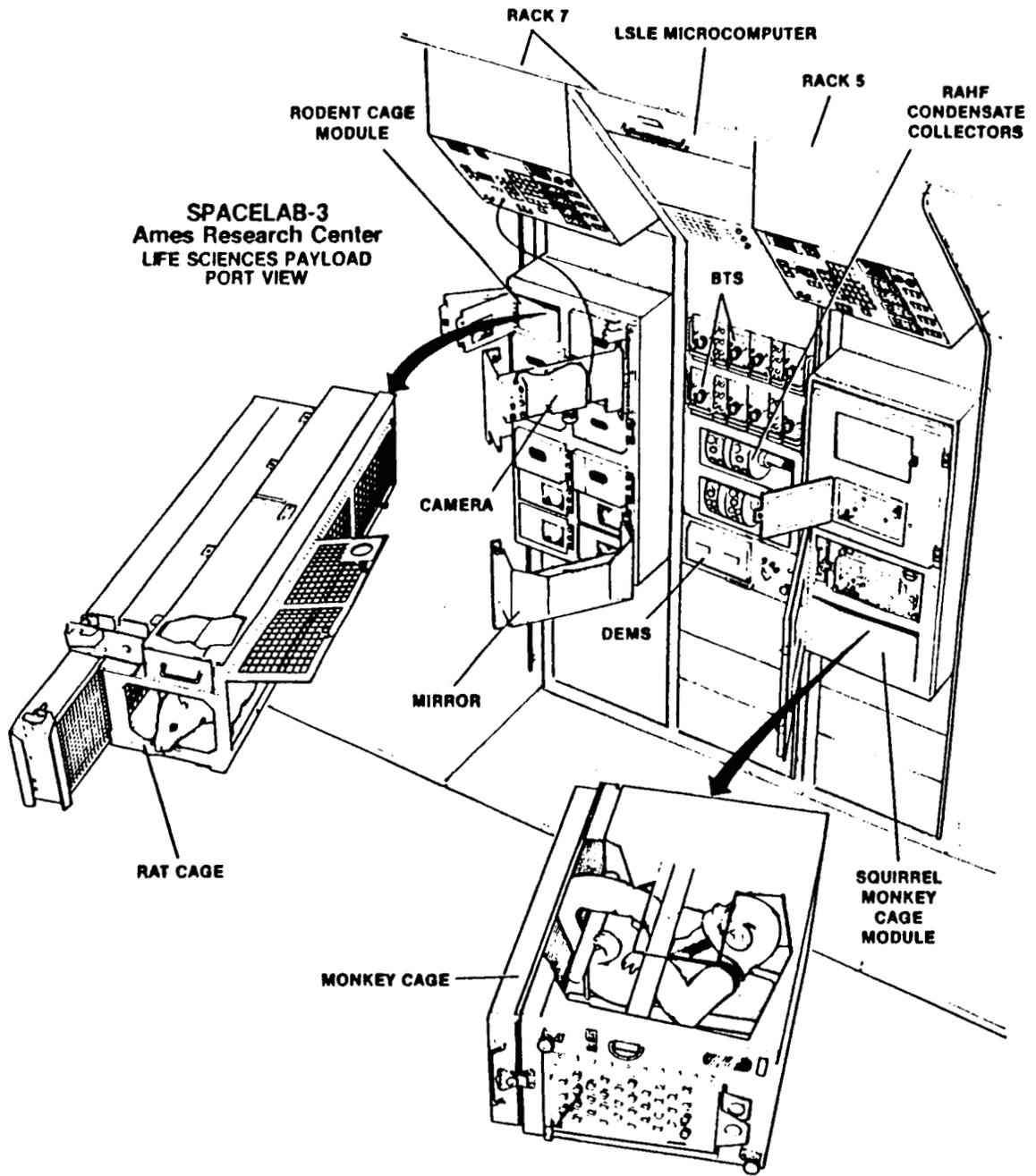


Fig. 10 Spacelab 3 life sciences payload.

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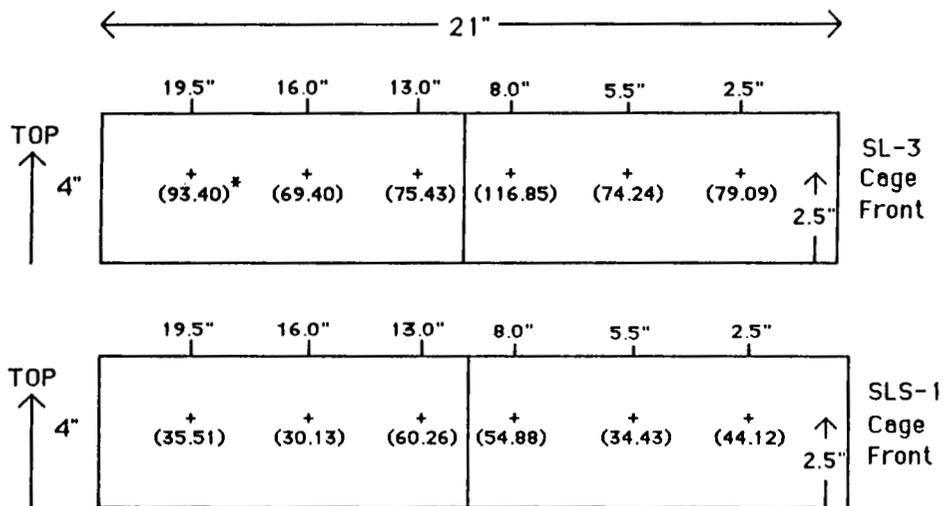


Fig. 11 SL-3 vs SLS-1 rodent cage.

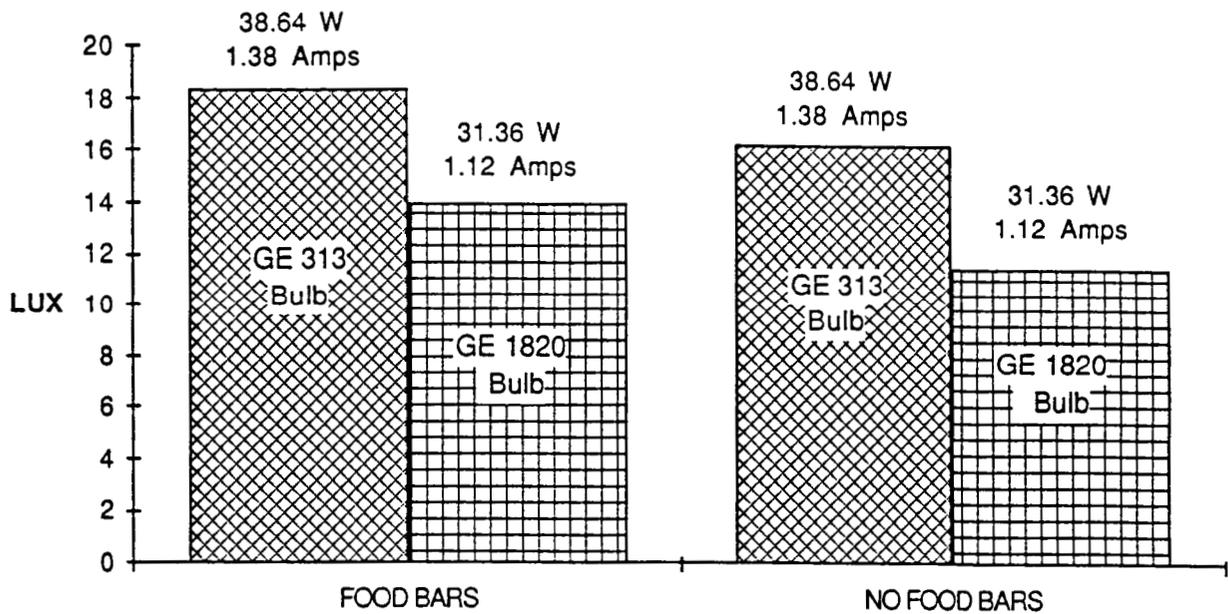


Fig. 12 AEM light level results: 28 VDC supply.

Measurement and Characterization of Light

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Light is generally defined as that portion of the electromagnetic radiation spectrum which can be detected by the human eye. This usually includes wavelengths from about 400 nanometers to about 750 nanometers. However, this is usually augmented by some of the ultraviolet wavelengths (200 to 400 nanometers) since they have such strong biological effects. In this definition we have already started discussing one of the primary measurement and characterization properties of light: it's spectral distribution. The other two properties which we will discuss are the geometric and temporal distribution of light and light sources.

FUNDAMENTALS

First, however, some of the fundamental properties of light should be mentioned. Optical radiation is propagated in sinusoidal waves. The wavelength is the linear distance between two similar points on adjacent periods. This distance is usually expressed in nanometers (one billionth of a meter). Light also has a particle character in addition to its wave character. The smallest discrete energy unit in which light can exist is the quantum or photon. The energy of the photon or quantum varies with frequency and wavelength according to Planck's law: $E = hv$.

The energy (E in ergs/second) of a photon is equal to the product of Planck's constant ($h=6.625 \times 10^{-27}$) and the frequency (ν). The particle nature of light is useful when considering the effects or detection of light at extremely low intensity levels.

Light intensity can often be described by one of two laws[1]. The inverse square law applies only to point light sources (see below). It states that the intensity of such a source varies inversely as the square of the normal distance to the source. (see Fig. 1a) The second law is the Lambert cosine law. It states that intensity varies directly with the cosine of the angle of incidence ($\cos \theta$) of the radiation. (Fig. 1b). Combining the two laws (Fig. 1c), if the distance to the source is expressed as the length of the normal from the source to the surface (h) rather than the straight linear distance (d), then the intensity varies as the cosine cubed.

PROPERTIES

The temporal characterization of light generally refers to either the long term (hours, days, etc.) time variation, which is usually externally controlled for biological experiments, or the short term (milliseconds) time variation, which is usually an inherent property of the light source - power supply combination. The long term variation is usually no problem since it is determined and therefore known by the experimenter. The short term variation can be a problem if it is not taken into account in the selection and operation of the detectors. The

most common examples are AC powered light sources which can have 60 or 120 Hertz flicker associated with their light output. False readings can result if these sources are measured with detectors that have a very short time constant. This can be corrected electrically either by integration or by AC synchronization of the detector.

The geometric characterization of light is complicated by the fact that there are no true point sources of light. An ideal point light source is defined as one whose geometric distribution is a perfect uniform radial distribution (spherical) and whose radiating surface dimensions are small compared to the distance from the source to the point of interest. Most artificial light sources are poor approximations to such an ideal source. This is particularly true when the reflected radiation from surrounding surfaces is taken into account. This can lead to very complicated spacial light distributions. That is the reason that most lighting situations are better characterized by the light impinging on a particular surface location rather than the light distribution of the source.

The spectral characterization of light is perhaps the most difficult because of the wide range of spectral distributions associated with common light sources. Although incandescent light sources result in a smooth well characterized spectral distribution (Planck's formula[2]), most other common light sources have a complex spectral distribution which is hard to

characterize simply (many sources also have superimposed spectral emission lines). This widely variable spectral distribution makes characterization very difficult, particularly since for biological applications one would like to characterize the distribution with as few numbers as possible. The currently accepted methods for this characterization will be described latter.

QUANTITIES AND UNITS

In most radiant energy problems the three quantities which are most often used are radiant power, radiance, and irradiance. Radiant power is usually used in measuring the total power (in watts) emanating from a particular light source. Radiance, however, is the power emanating from a source per unit area of source surface and per unit solid angle (in steradians) in a particular direction. Although by definition radiance can also be used for incoming power as well, it is most commonly used for outgoing power. Irradiance is always used to measure incoming radiation. It is the total radiant power incident upon a surface from all directions per unit area of surface.

Electromagnetic radiation is quantitatively expressed in radiometric and International System (SI) units. The radiant power is in watts, the radiant energy is in joules, the irradiated area is in meters², and the irradiated volume is contained in the unit solid angle (in steradians). The units[3] are summarized in Table I. The modifier, spectral, may be added

to any of these units to indicate that the measurement refers to a specific spectral band or wavelength. The most often used quantities are the (total) radiant power of a light source, the irradiance at a surface location, and, to a lesser extent, the radiance of a light source in a particular direction.

PHOTOMETRICS

The international standardizing authority, The Commission Internationale de l'Eclairage(CIE), has standardized the relative spectral sensitivity functions for human vision. This is given in Figure 2 as two curves[3]. The standard photopic curve is for the fully light-adapted human observer and the standard scotopic curve is for the fully dark-adapted human observer. Photometric quantities express the corresponding radiometric quantities with energy or power at each wavelength weighted by the standard photopic curve before summation over wavelength. This results[3] in photometric quantities shown in Table II with its units involving lumens rather than watts. Thus radiant power becomes luminous flux or power and is measured in lumens. Radiance becomes luminance and is measured in lumens per square meter per steradian. Finally, irradiance becomes illuminance and is measured in lumens per square meter.

SPECTRAL CHARACTERIZATION

The spectral dispersion of a light source or lighting situation is usually the most difficult characteristic to

quantify. The most straight-forward and tedious method is simply to show the entire spectral power distribution (SPD). However, even this is not as simple as it appears since a bandwidth has to be chosen for the display. This is no problem for incandescent light sources which have slow, smoothly varying distributions [3] (Fig. 3). However, for sources which contain spectral emission lines (eg. fluorescent lamps) the choice of bandwidth can make a large difference in the appearance of the SPD. Figure 4 shows an example of the SPD of a cool white fluorescent lamp plotted with a bandwidth of 10 nanometers. In addition to using a uniform bandwidth, various systems have been used which assign wavelength ranges to a specific set of color names, usually based on tests of color discrimination of a group of human observers. Figures 5 and 6 show this same cool white lamp plotted according to a European eight color system and an American six color system, respectively. It can be seen that all of the curves in Figures 4 through 6 look quite different.

In the attempt to quantify wavelength and color characterization, the CIE has defined the 1931 standard observer [1]. This is shown in Figure 7 as three spectral curves, an X, Y and Z. Although there are many other standard observer color matching systems, this seems to be the one in widest use. It was determined originally by matching experiments on a trichromatic colorimeter [3]. It attempts to represent a standardized human response to color and is used by multiplying each curve in turn by the SPD of a light source, wavelength by

wavelength, and then summing over all wavelengths for each curve. The X, Y, and Z values thus obtained are called the tristimulus values. In order to be able to obtain two dimensional graphs of these values, chromaticity coordinates x , y and z are defined by adding the normalizing condition that the three values add up to 1. The x and y chromaticity coordinates can then be plotted[1] as in Figure 8. Every point on this plot is represented by a particular color appearance and every SPD can be represented by a point on this plot. However, care must be taken in interpreting this plot because it is not true that every point on this plot is represented by a particular SPD. A multitude of SPD's can be represented by the same point on the plot. Although this means that the chromaticity coordinates are not a complete characterization of the SPD, it is still a useful and widely used characterization scheme.

Another SPD characterization can be obtained if the SPD of black body radiators at different temperatures (ie. the color temperature) are plotted on the chromaticity coordinate diagram. The locus of these points are shown in Figure 8. If points on either side of this locus are correspondingly associated with one of these color temperatures, this is then the correlated color temperature of each of these points. This association is shown in Figure 9 which shows the lines of constant correlated color temperature for points on each side of the black body locus[2]. Although the correlated color temperature is an excellent characterization for incandescent light sources, it

must be combined with other characterization methods to be useful for other types of light sources.

The final spectral characterization method which will be discussed is one that approaches the problem from the point of view of how a particular light source renders an array of carefully chosen colors. The fourteen CIE colors have been chosen to try to cover the various areas of the chromaticity coordinate diagram and are specified by their spectral reflectances[1]. For any light source of a particular correlated color temperature its color rendering of these fourteen colors is compared against that of an "ideal" light source of the same color temperature. Below color temperatures of 5000 degree Kelvin, the ideal source is the black body radiator. Above 5000 the ideal source is a standardly defined daylight equivalent light source. The comparison is done by calculating the differences in the chromaticity coordinates of the light reflected by each source from each of the colors. The resulting color rendition index (CRI) is 100 if the chromaticity coordinates of all of the colors used are equal for either light source. The CRI decreases as the corresponding chromaticity coordinates get farther apart. The CRI quoted by most lighting companies uses only the first eight CIE color samples and is correctly referred to as the CRI-A. The CRI using the last six CIE color samples is called the CRI-B. However, the overall CRI is still the better characterization measure of a light source. For example, Table III gives the approximate CRI, CRI-A, and

CRI-B for several common fluorescent light sources.

MEASUREMENT - DETECTORS

In any light measurement technique an appropriate detector is crucial to success. The most important performance characteristics of any detector are overall sensitivity, relative spectral sensitivity, spatial response, stability, linearity, and time constant. Most modern detectors are either thermoelectric or photoelectric. Thermoelectric detectors use a blackened surface to absorb the radiation to be measured, thus raising its temperature. This temperature rise is then measured with either thermocouples, thermopiles, or the temperature coefficient of resistance of the blackened substrate. The main advantage of thermoelectric detectors is their relative insensitivity to wavelength. They have a much longer time constant than most other detectors. Based on most important detector characteristics, photoelectric detectors are usually a better choice and are thus in more general use.

Photoelectric detectors are those in which the incident radiation directly causes an electronic effect. They all exhibit a strong wavelength dependent sensitivity (a sample of the spectral response of some of the more popular types of photoelectric cells[3] are shown in Figure 10). According to the type of electronic effect which is used, these detectors fall into three broad categories: photovoltaic, photoconductive, and photoemissive.

In photovoltaic cells the incident radiation directly produces current or voltage in an external passive circuit. They are most linear when generating current into a low impedance circuit. Selenium cells are commonly used because their wavelength response is close to that of the photopic eye response curve and can be made even closer with a properly designed filter. Silicon cells (which are the most popular) are more stable than selenium cells and can be made to have a reasonably photopic response with appropriate filtering. Both types of cells have high sensitivity.

The absorbed radiation in photoconductive cells results in a change in the conductivity of the cell which is measured using an externally powered circuit. They are characterized by very high sensitivity and very short time constants. However, care must be taken regarding their linearity and hysteresis effects. Photodiodes and phototransistors are also included in this category. These detectors are notable because their high sensitivity and modern microfabrication methods have resulted in their being made into detector arrays for fast scanning spectrometers and position sensitive detectors.

Finally, in photoemissive detectors the absorbed radiation results in the emission of electrons from a cathode (Figure 10). In vacuum type cells these electrons are collected across a vacuum by a positively biased anode. If a series of increasingly biased intermediate electrodes are used to collect and amplify the photoemitted electrons, the result is a photomultiplier

tube. These detectors have the very highest sensitivity which makes them useful for narrow bandwidth applications, such as spectrometer detectors.

A note of caution is in order concerning the very short time constants available in some of these photoelectric detectors. When alternating current (AC) based light sources are used which have a residual AC component to their light output, these detectors can produce a misleading output due to variations in the sampling of the temporal signal, since their time constant is less than that of the AC light component time constant. Even when photopicly corrected, they will not correspond to one's eye perception because of the eye's longer time constant. However, when necessary, the time constant of the photoelectric detectors can be electronically lengthened by integration.

MEASUREMENT - INSTRUMENTS

Most instruments which are used to measure optical radiation can be classified by how they handle three measurement characteristics: the geometric distribution of the radiation, the wavelength dependence of the radiation, and the measurement or calibration technique.

The geometric distribution is most commonly handled by one of three techniques. A diffusing dome over the detector can provide the cosine correction needed for illuminance or irradiance instruments. An optical system can be used in front of a detector in order to define the radiation acceptance angle for

luminance or radiance instruments. An integrating sphere (which is lined with a diffusing, nonselectively reflecting, nonabsorbing coating) can be used in a variety of ways for geometric integration or averaging. It is most commonly used to measure the total light flux from a light source.

The wavelength dependence of light is usually handled by instruments in one of three ways. A monochromator can be used to select or scan wavelength. These instruments usually have the prefix, spectro, attached to them. Secondly, the detector can be filtered to match the eye response sensitivity curve. This type of instrument usually has the designation, photo or lumen, in its title. Finally, the detector can be interference or absorption filtered to only accept radiation in a certain wavelength range for special purposes (eg. UVA and UVB detectors).

The measurement or calibration technique[3] usually determines the units of measurement, the accuracy and precision of the measurement and the ultimate usefulness of the measurement. If the calibration results in a measurement in watts, the instrument can be called a radiometer. If an integrating sphere with a photopically corrected detector is calibrated against a lamp of known lumen output and is then used to measure a lamp of similar type, then the instrument is called a photometer. Equivalent sphere illumination (ESI) photometers use a type of integrating sphere and a photopically corrected detector. However, the instrument becomes an ESI device from the

measurement technique of comparing a task viewed in an actual environment to the same task viewed in the luminous sphere. Another example is the reflectometer, which is basically a photometer with a slightly different physical arrangement and a measurement technique that results in the determination of the reflectivity of materials or surfaces.

With all of these measurement techniques and instruments it is important to keep in mind the exact specifications and limitations of each of the above components so that the investigator measures what he/she thinks he/she is measuring. Unfortunately, it is probably easier to make this kind of error in the field of light and its biological effects than in most other fields.

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TABLE I. Fundamental Radiometric Quantities (3)

| Quantity | Symbol | Defining equation | Unit | Symbol |
|--|----------------------|--|---|---|
| Radiant energy | Q, (Q _e) | | Erg Joule Calorie Kilowatt-hour | erg J cal kWh |
| Radiant energy density | w, (w _e) | w = dQ/dV | Joule per cubic meter Erg per cubic centimeter | J/m ³ erg/cm ³ |
| Radiant flux | Φ, (Φ _e) | Φ = dQ/dt | Erg per second Watt | erg/s W |
| Radiant flux density at a surface | | | | |
| Radiant excittance (radiant emittance) | M, (M _e) | M = dΦ/dA | Watt per square centimeter, Watt per square meter, etc. | W/cm ² W/m ² |
| Irradiance | E, (E _e) | E = dΦ/dA | | |
| Radiant intensity | I, (I _e) | I = dΦ/dω (ω = solid angle through which flux from point source is radiated) | Watt per steradian | W/sr |
| Radiance | L, (L _e) | L = d ² Φ/(dωdA cos θ) = dI/(dA cos θ) (θ = angle between line of sight and normal to surface considered) | Watt per steradian and square centimeter Watt per steradian and square meter | W/sr·cm ² W/sr·m ² |

TABLE II. Fundamental Photometric Quantities (3)

| Quantity | Symbol | Defining equation | Unit | Symbol |
|--|----------------------|--|--|--|
| Luminous efficacy | K | K = Φ _v /Φ _e | Lumen per watt | lm/W |
| Luminous efficiency | V | V = K/K _{maximum} [K _{maximum} = maximum value of K(λ) function] | One (numeric) | |
| Luminous energy (quantity of light) | Q, (Q _v) | Q _v = ∫ ₃₈₀ ⁷⁷⁰ K(λ) Q _{eλ} dλ | Lumen-hour Lumen-second (talbot) | lm·h lm·s |
| Luminous energy density | w, (w _v) | w = dQ/dV | Lumen-hour per cubic centimeter | lm·h/cm ³ |
| Luminous flux | Φ, (Φ _v) | Φ = dQ/dt | Lumen | lm |
| Luminous flux density at a surface | | | | |
| Luminous excittance (luminous emittance) | M, (M _v) | M = dΦ/dA | Lumen per square foot | lm/ft ² |
| Illuminance (illumination) | E, (E _v) | E = dΦ/dA | Footcandle (lumen per square foot) Lux (lm/m ²) Phot (lm/cm ²) | fc lx ph |
| Luminous intensity (candle power) | I, (I _v) | I = dΦ/dω (ω = solid angle through which flux from point source is radiated) | Candela (lumen per steradian) | cd |
| Luminance | L, (L _v) | L = d ² Φ/(dωdA cos θ) = dI/(dA cos θ) (θ = angle between line of sight and normal to surface considered) | Candela per unit area Stilb (cd/cm ²) Nit (cd/m ²) Footlambert (cd/πft ²) Lambert (cd/πcm ²) Apostilb (cd/πm ²) | cd/in ² , etc. sb nt, cd/m ² fL L asb |

Table III Color Rendition Index for some common fluorescent lamps

| Source | CRI | CRI-A | CRI-B |
|------------|-----|-------|-------|
| D5500 | 100 | 100 | 100 |
| Vitalite | 88 | 91 | 84 |
| Triband | 73 | 83 | 60 |
| Cool White | 44 | 62 | 20 |

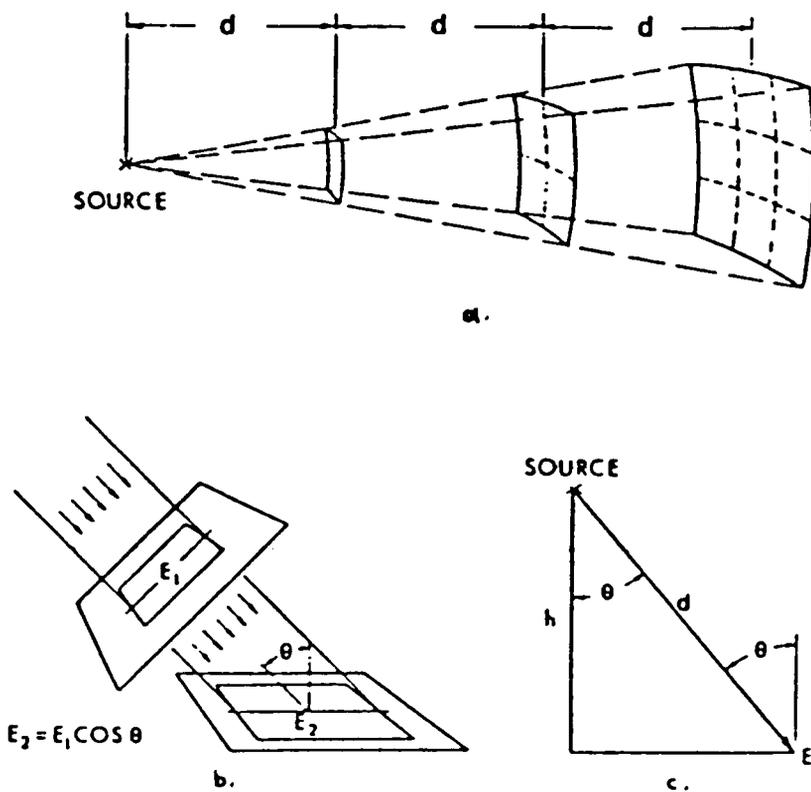


Fig. 1 a. The inverse-square law. b. The Lambert cosine law. c. The cosine-cubed law. (1)

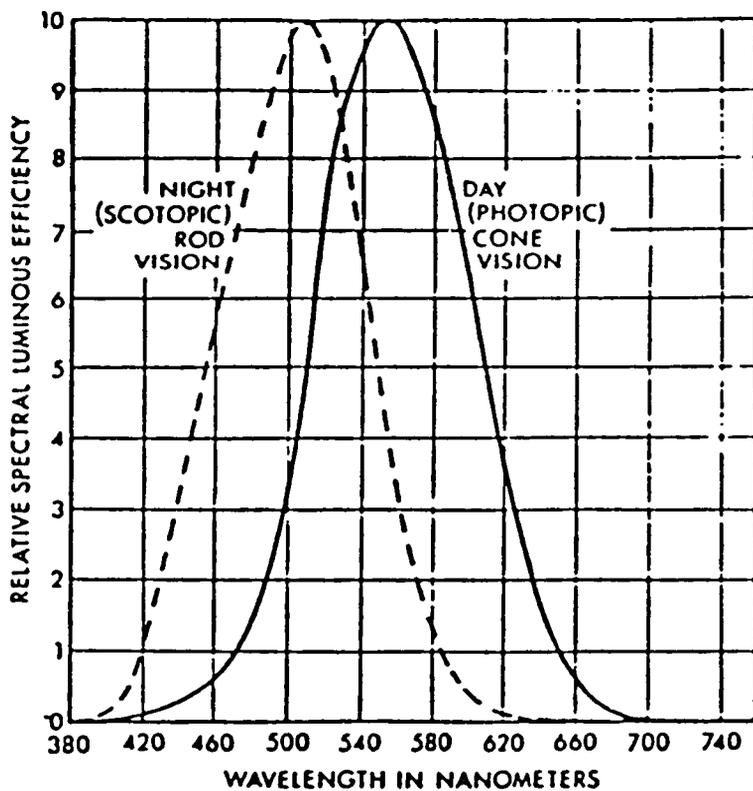


Fig. 2 The relative spectral sensitivity for photopic (cone) and scotopic (rod) vision. (3)

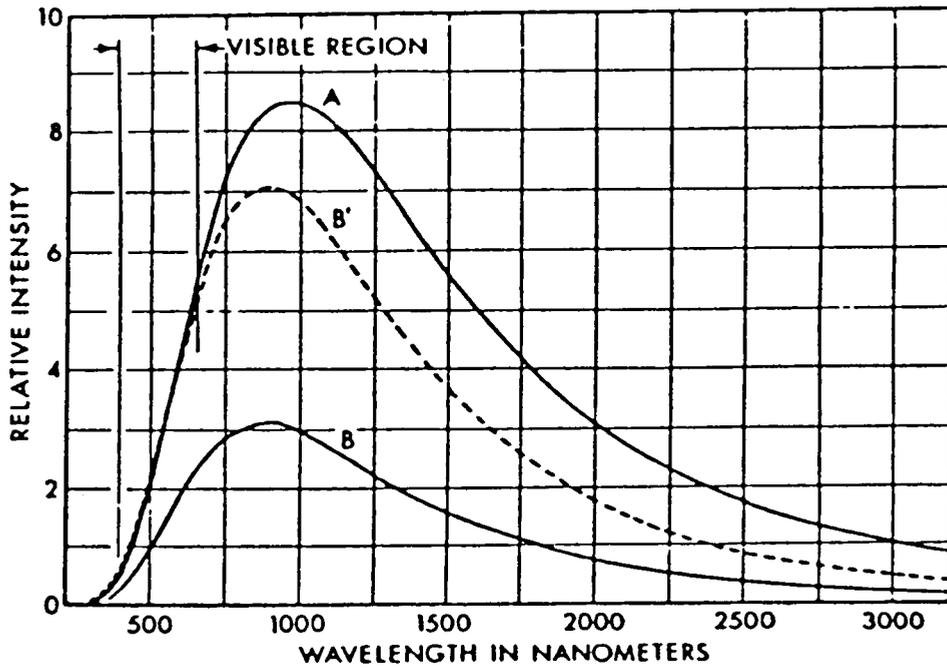


Fig. 3 Radiating characteristics of tungsten. Curve A: Radiant flux from 1 cm² of a blackbody at 3,000°K. Curve B: Radiant flux from 1 cm² of tungsten at 3,000°K. Curve B': Radiant flux from 2.27 cm² of tungsten at 3,000°K (equal to curve A in visible region [Kaufman and Christensen, 1984]). (1)

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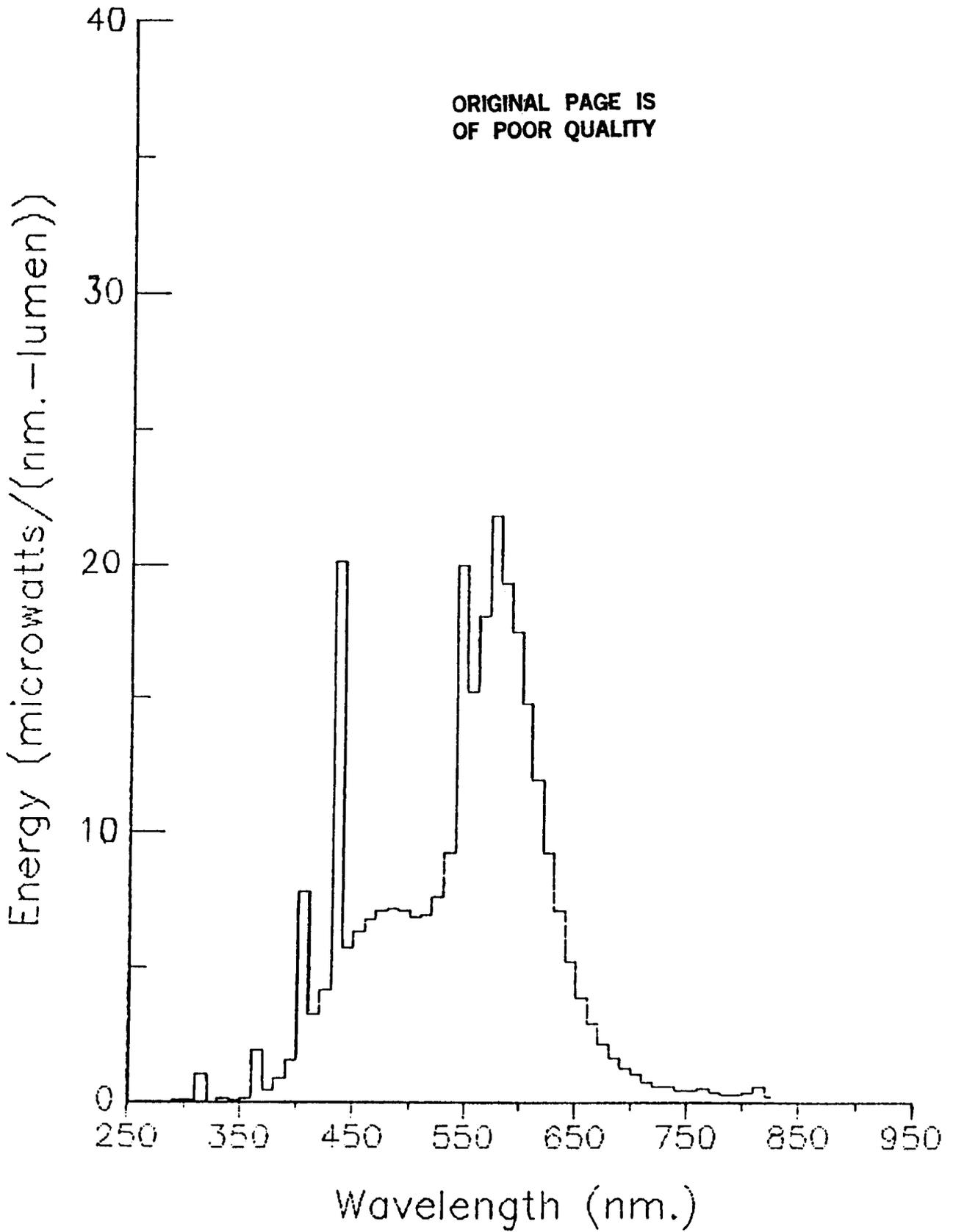


Fig. 4 Spectral power distribution of cool white fluorescent lamp using 10 nm bandwidth.

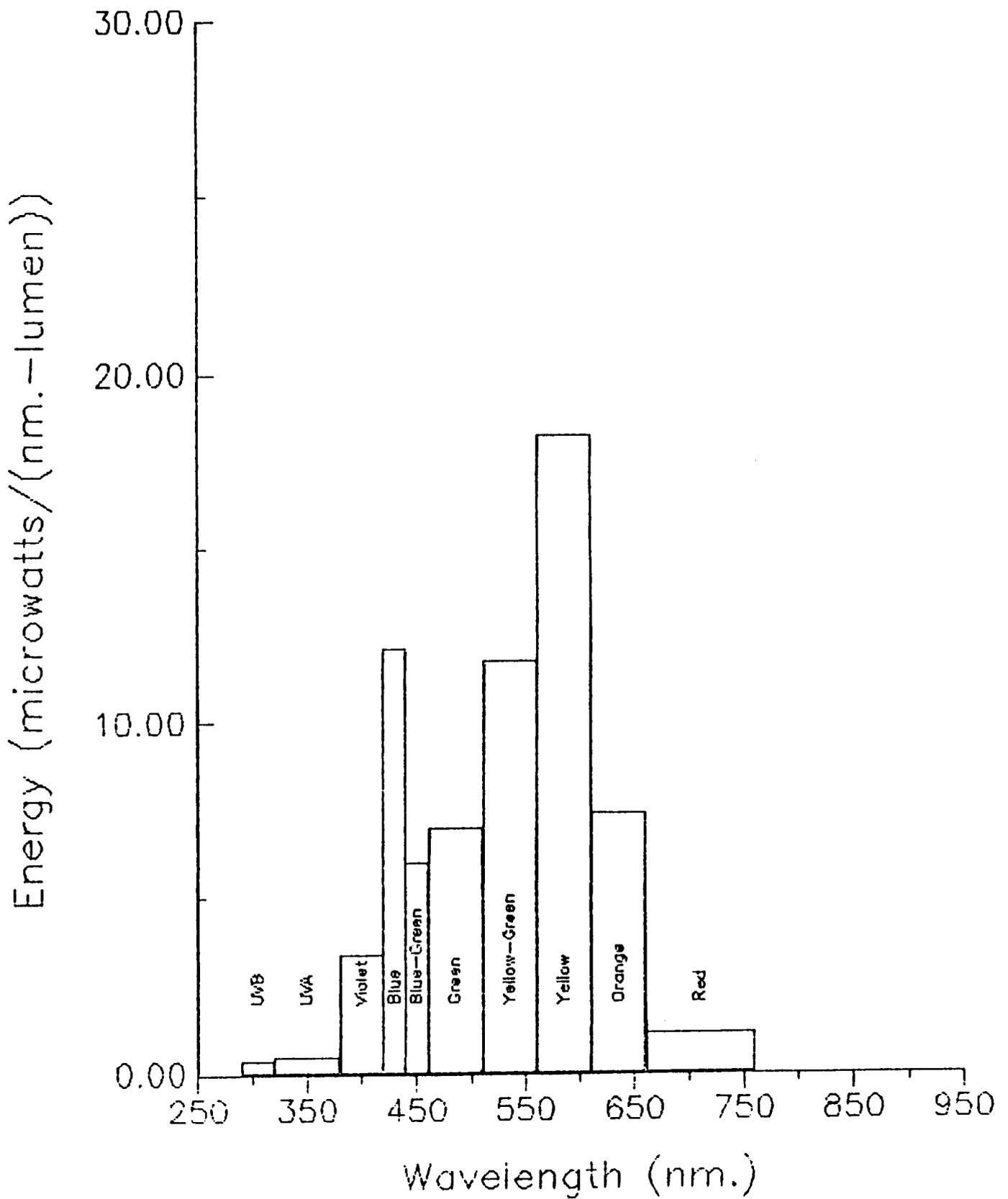


Fig. 5 Spectral power distribution of cool white fluorescent lamp using European 8 color system.

LAMP: LT0338

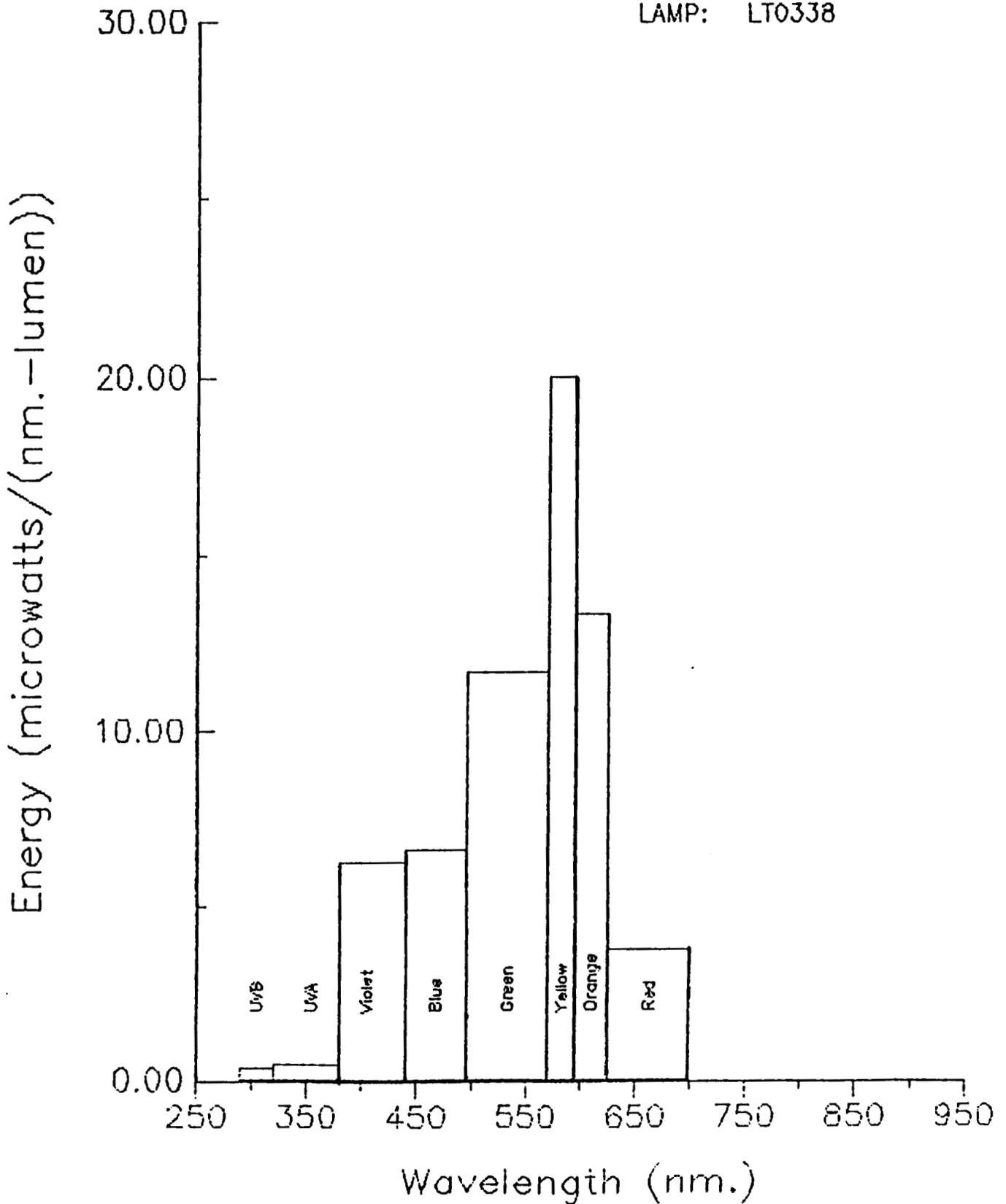


Fig. 6 Spectral power distribution of cool white fluorescent lamp American 6 color system.

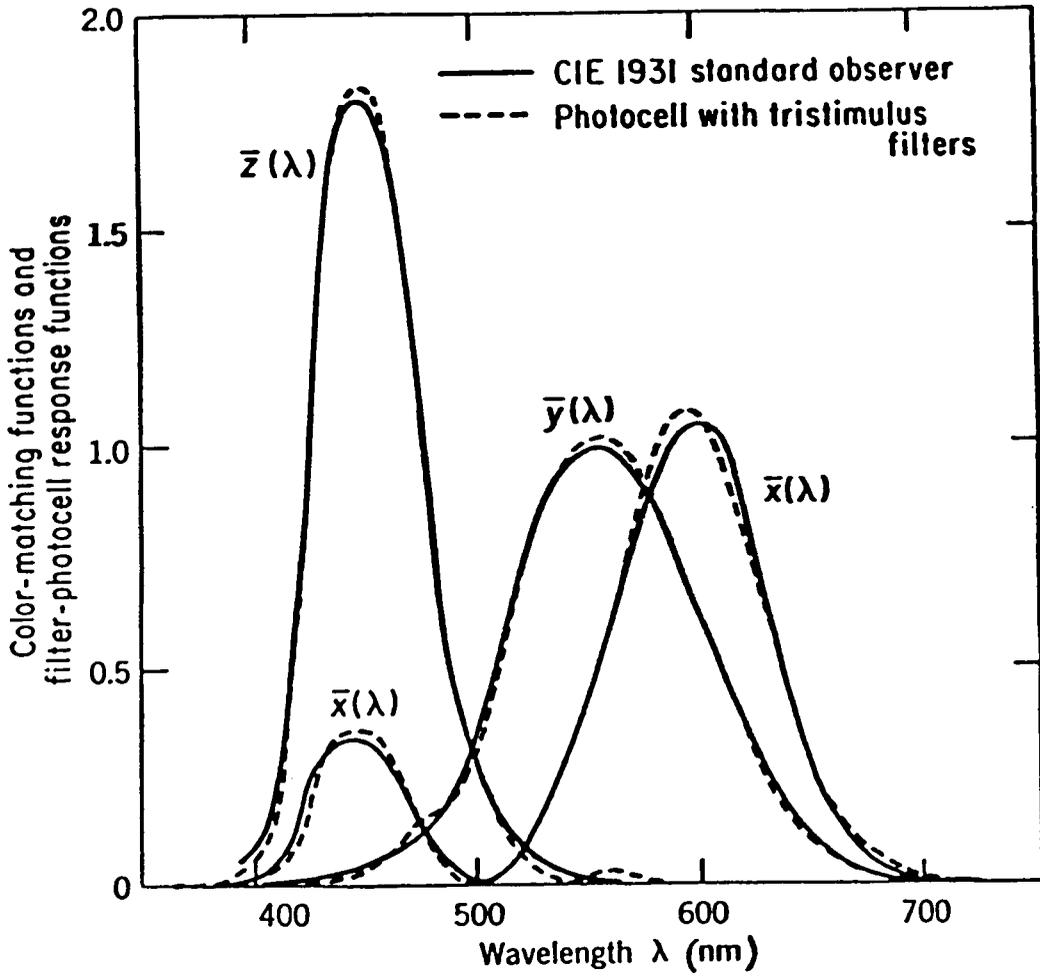


Fig. 7 CIE 1931 tristimulus response. (2)

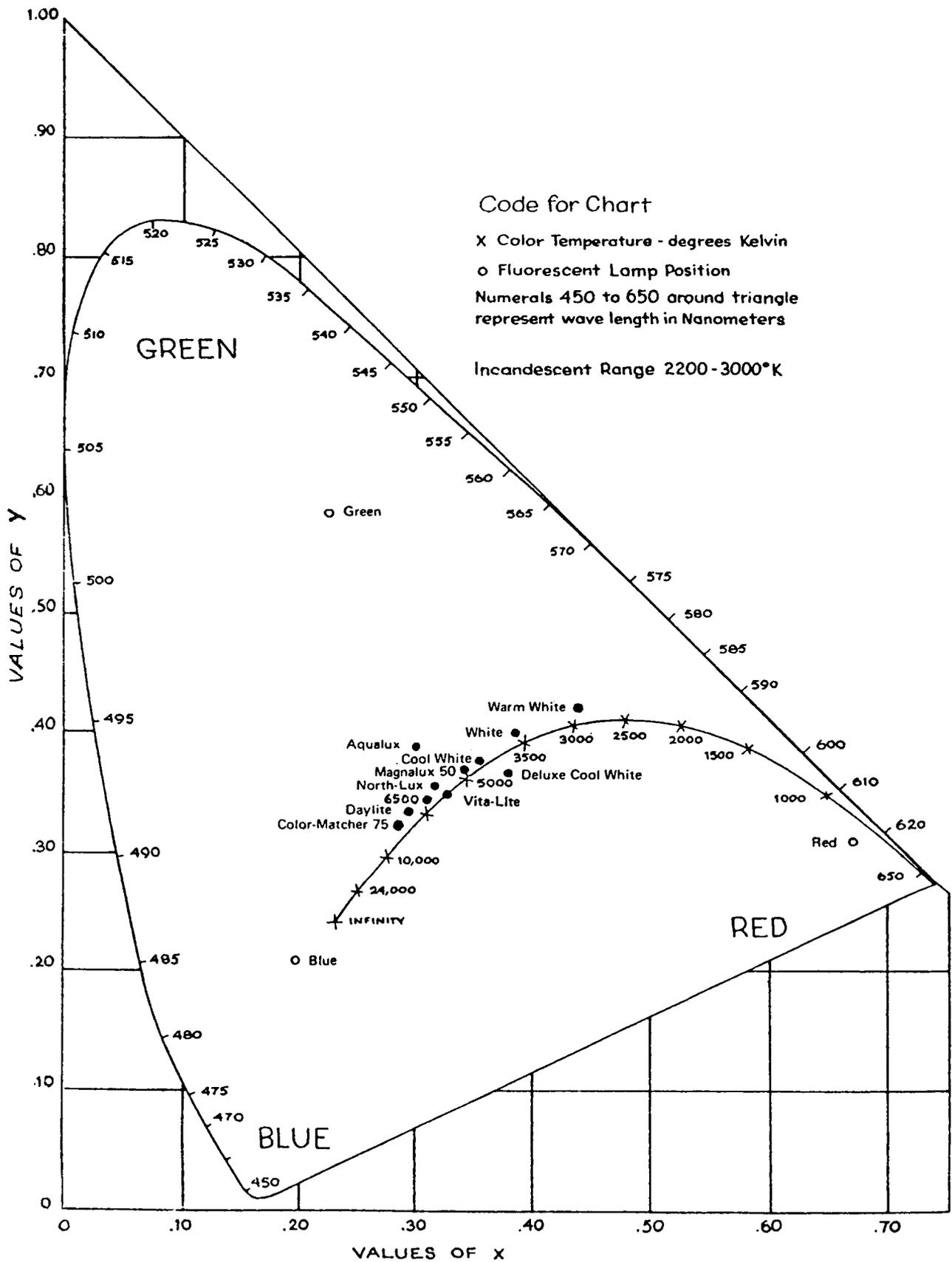


Fig. 8 Chromaticity diagram based on C.I.E. system (International Commission on Illumination).

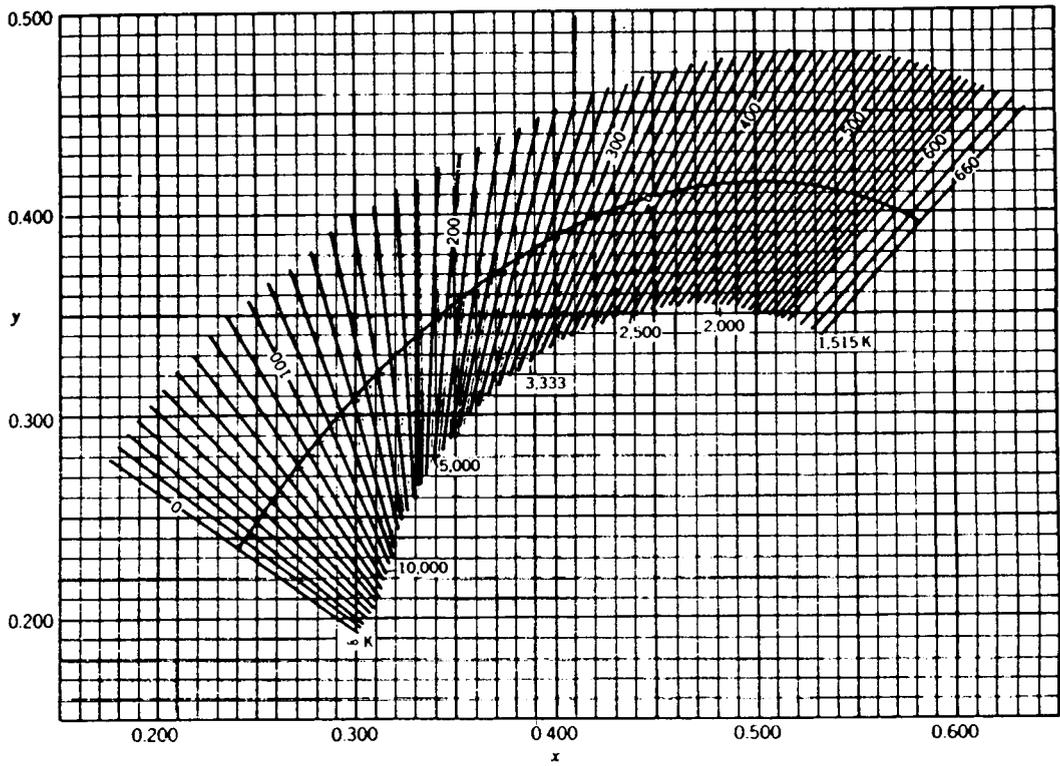


Fig. 9 CIE 1931 chromaticity diagram showing isotherm lines. (2)

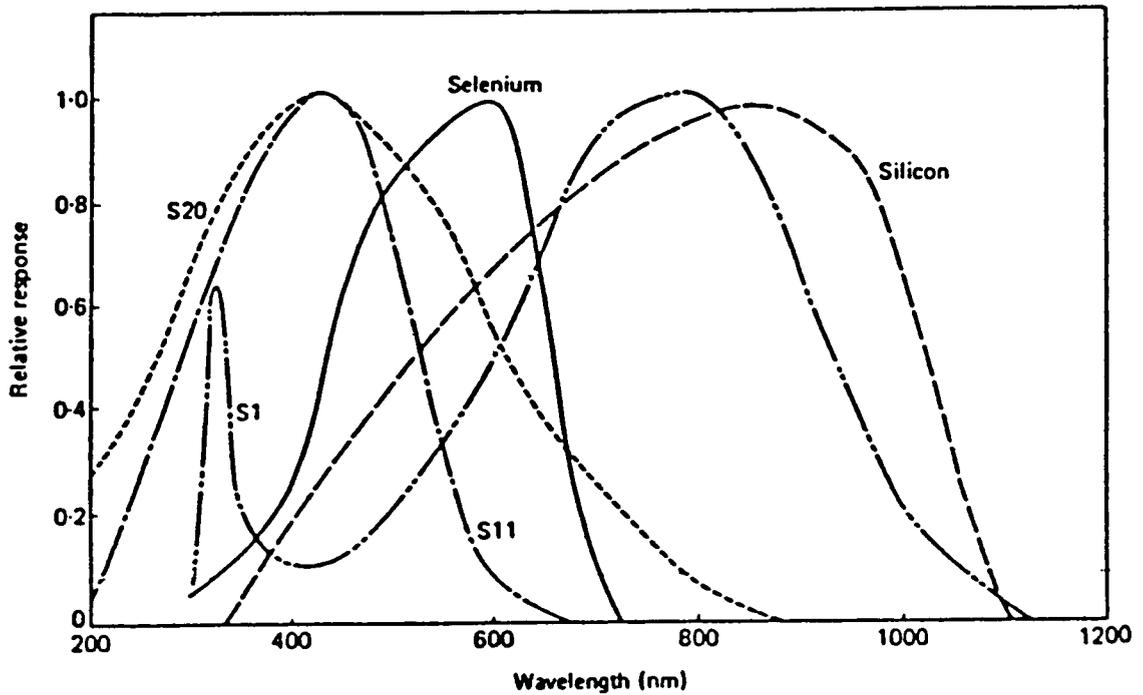


Fig. 10 Relative spectral response curves of selenium and silicon photovoltaic cells and three photocathode materials AgOCs (S1), CsSbo(S11), and NaKSbCs (S20). (3)

LIGHT SOURCE CHARACTERISTICS AND SELECTION

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INTRODUCTION

There are many ways to get good lighting for animal habitats. The purpose of this lecture is to discuss the characteristics of various types of light sources and the factors which influence the selection of one light source over another. The general characteristics of light sources can be obtained from the Illuminating Engineering Society (IES), Reference and Application handbooks.(1)

It is my purpose in this paper to build on the IES background material and simplify, as far as possible, the selection of a light source. For any given lighting system the lighting designer has a wide range of types and sizes of light sources, luminaries and lighting equipment. The task required of the lighting system, the economics, and the coordination of the mechanical environmental systems are three considerations which are important in the selection of the light source. Electric light sources are principally developed for human seeing. Most humans can see a wide range of light in the visible spectrum with the greatest sensitivity in the yellow-green region. Animals can also see a wide range of color, but their particular spectral response versus wavelength is often different than a human being. This needs to be taken into account in the selection of a light source for a particular animal.

The types of light sources that are generally considered today are incandescent, tungsten halogen, fluorescent, metal halide and high pressure sodium. Generally, for animal habitats where low light levels and flexibility are required, incandescent lamps or fluorescent lamps are preferred.

CHROMATICITY

It is frequently useful to use a chromaticity diagram (2) which displays all the possible colors of the rainbow on a convenient chart. A color of a particular light source can then be specified by the "XY" coordinates or often more simply in Kelvins on a black body curve, also positioned on the diagram. Incandescent lamps have a smooth continuous spectrum as shown in Figure 1. Other light source spectrum are more complex, nevertheless, it is helpful to refer to Kelvins with these light sources extending the concept to a correlated color temperature. Light sources for general seeing purposes tend to be in the white area of the chromaticity diagram and these light sources are designed to be as close to the black body locus as possible. A broad spectrum source somewhat duplicating daylight and sunlight will have a correlated color of about 5000K. Incandescent lamps or tungsten halogen lamps, on the other hand, lack blue intensity and will have a warmer correlated color temperature of approximately 3000K.

SPECTRAL POWER DISTRIBUTION CURVES

As shown in the illustrated part of this lecture, each light source can be broken up into a spectral profile of the visible colors plus any infrared radiation or ultraviolet radiation. This profile is a spectral power distribution curve and is usually given over the visible range of wave lengths from 400 to 700 nanometers. Fluorescent lamps tend to have a combination of continuous and broad band spectra as shown in Figures 2 and 3. The popular fluorescent cool white is shown in Figure 2 and a fluorescent lamp closely matching sunlight in Figure 3. The high intensity discharge lamps, either metal halide, mercury or high pressure sodium tend to produce light in discreet bands or lines and produce very special power distribution curves. Before specifying a particular lamp light source it is important to compare this light source with daylight and with the visual response of a particular animal. It is difficult to describe daylight as a spectral power distribution since the light spectrum is constantly changing. Figure 4 shows the spectrum for two common conditions (3). Correlated color temperatures from 5000K to 6500K are representative of daylight.

SPECIFYING A LIGHT SOURCE

The first requirement in specifying a particular lamp and lighting system is to determine the task required. Often for animal habitats, low lighting levels (lux) are adequate. The next requirement is to determine if a specific color or spectral power distribution is required. If this is not important for the task requirement, incandescent lamps may be adequate. Another energy efficient choice is fluorescent, possibly cool white design. If there is a desire to match the sun spectrum more closely, choose a fluorescent lamp with a correlated color temperature of about 5000K, close to the sun's spectrum. The non-visible portions of spectrum (ultra violet or the infrared) from electric light sources usually have low intensity compared to daylight. Therefore, it is generally assumed that these are not a factor in the selection of the light source.

There are certain engineering requirements to keep in mind in selecting a light source, in that all discharge light sources require an auxiliary circuit to operate them satisfactorily. Incandescent light sources are the simplest in the terms of circuit requirements.

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2. IES Reference Volumes, page 5-9, Figure 5-7.
3. A.H. Taylor and G.P. Kerr, The distribution of energy in the visible spectrum of daylight, Journal of the Optical Society of America 31,3 (1941).

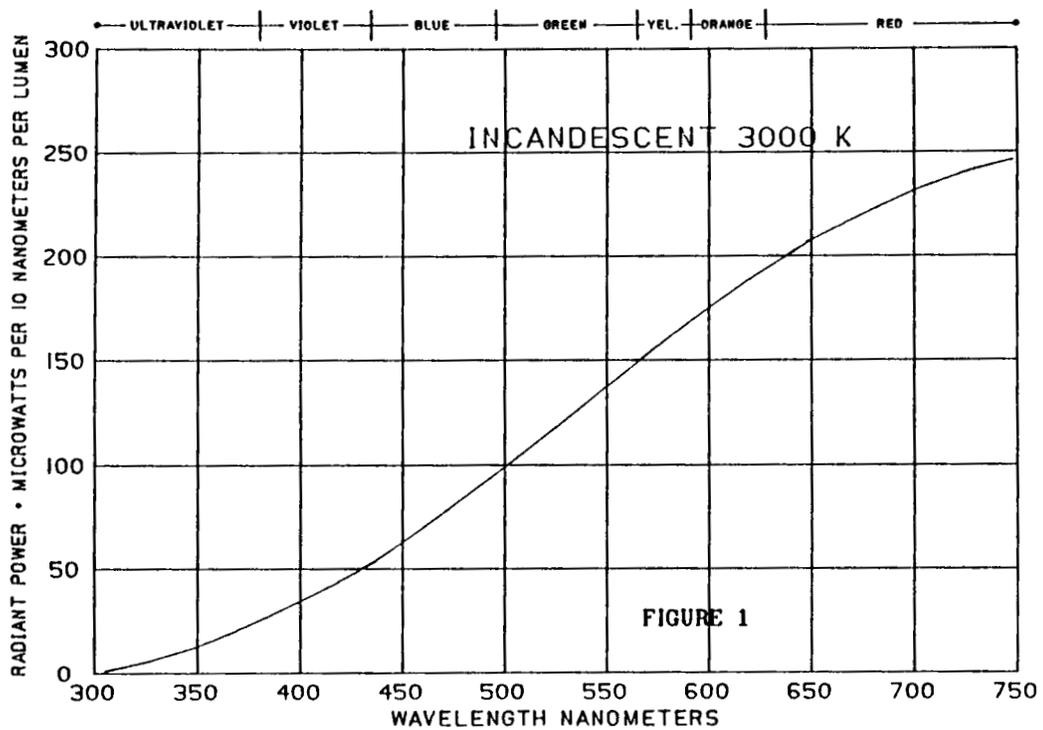


Fig. 1 Spectral energy distribution of incandescent lamp.

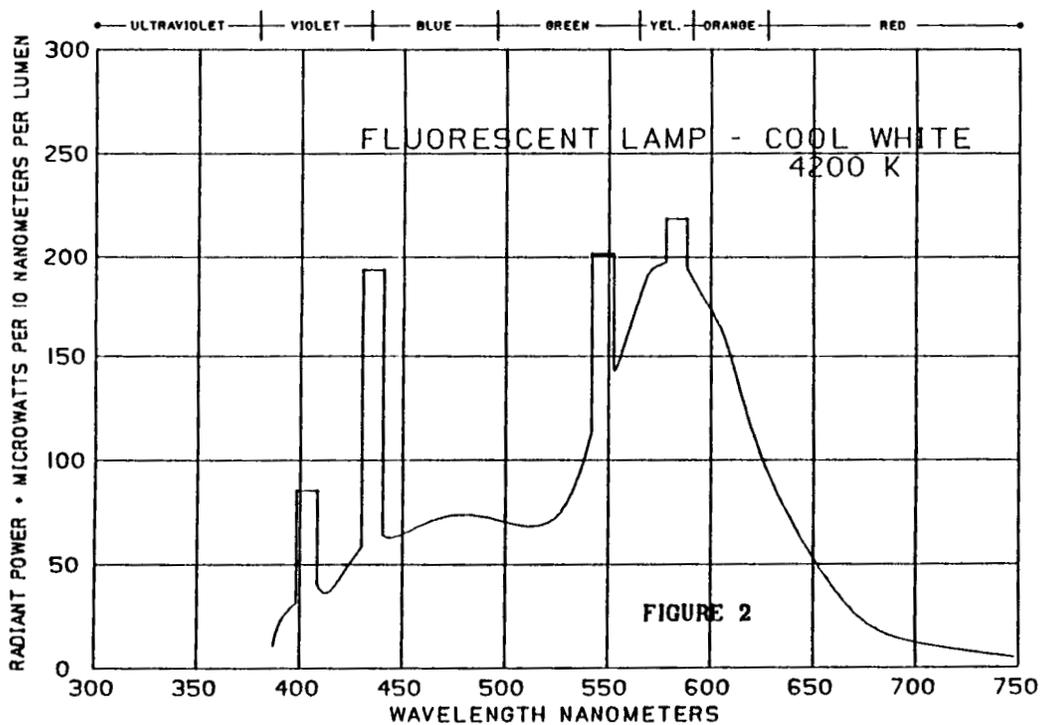


Fig. 2 Spectral energy distribution of fluorescent lamp--cool white.

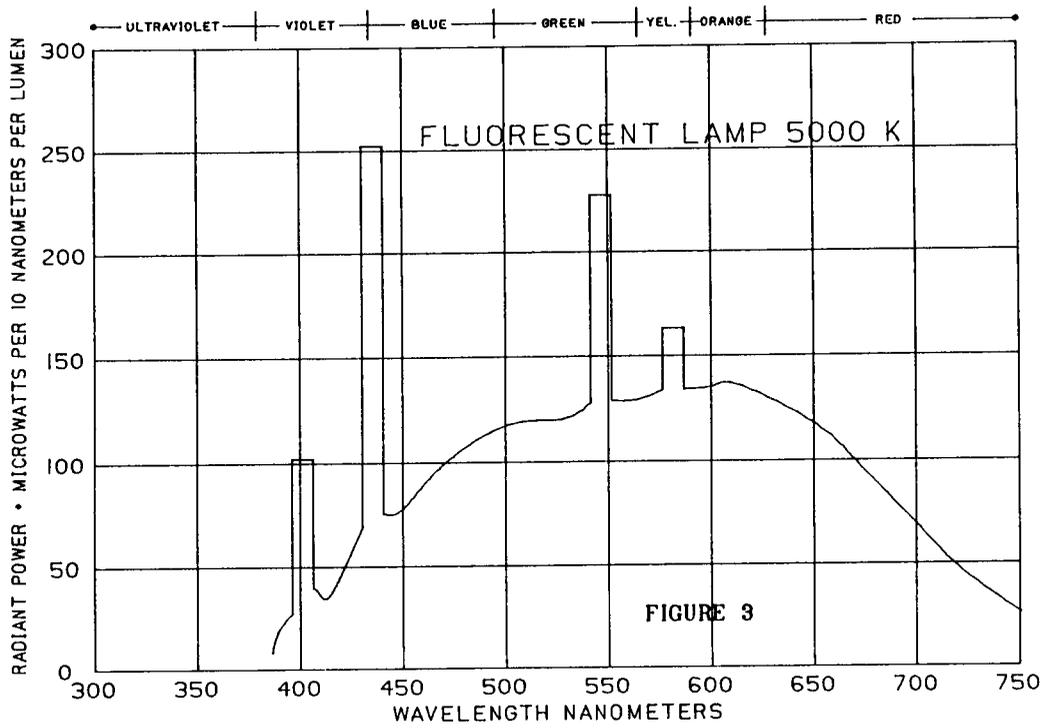


Fig. 3 Spectral energy distribution of fluorescent lamp (5000°K).

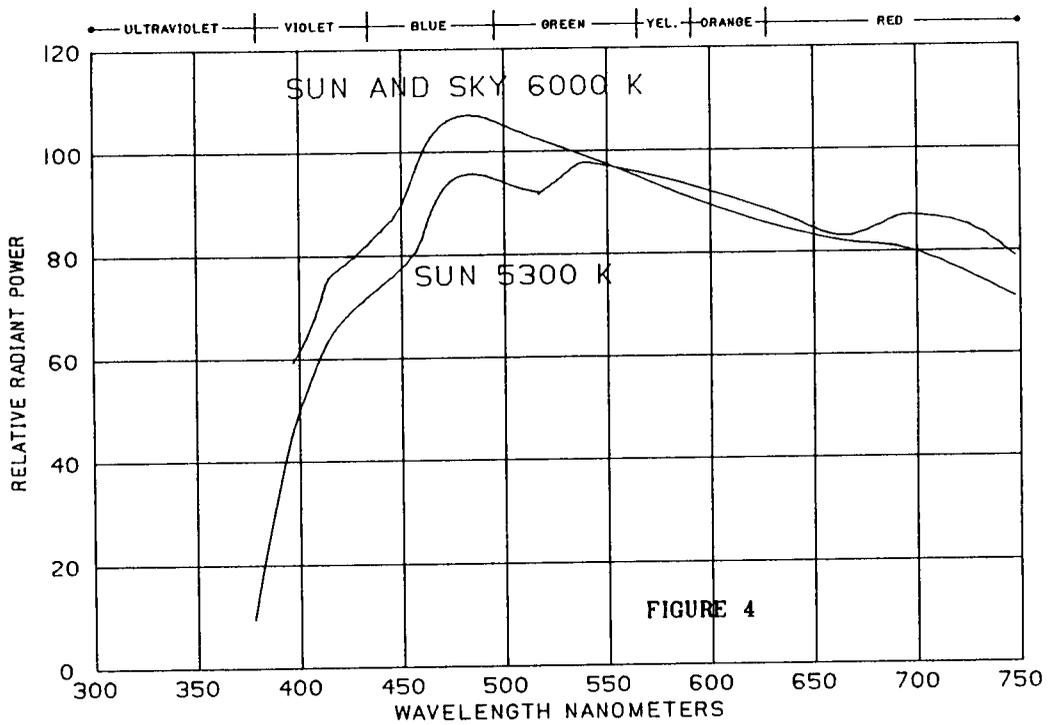


Fig. 4 Spectral energy distribution of Sun and sky.

AVAILABLE SPACE QUALIFIED LIGHT SOURCES

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I would like to describe some of the space qualified light sources that presently exist. By space qualified I do not mean to imply that lights presently used in animal experiments are not space qualified. What I mean by space qualified are principally shuttle lamp fixtures that are actually an integral part of the spacecraft. They are qualified to a different level than lights that go on experiments. Also, I should tell you that the lights I am going to describe are probably higher wattage than are applicable to many of the animal experiments being considered.

There are three purposes for describing these lights. One is to give you a feeling for what some of the shuttle fixtures look like and what performance characteristics they have. Second, by studying these existing fixtures we can minimize the effort in developing new light sources for particular experiments. Third, there is the odd chance that one of the existing lights may be applicable to an existing experiment. However, these lights are fairly expensive. The average price might be \$20,000 for a space qualified fixture.

(Figure 1) All of the interior general lighting fixtures on the shuttle are fluorescents. Almost all task lighting is also fluorescent. These fluorescent tubes are specially manufactured and are not standard or catalog items. This is partly because of the space requirements for traceability back to the raw material and partly because the launch requires such special items as reinforced filaments and special care in the lamp manufacture. Up to 24 different interior assemblies exist. A number of these are just very slight variations from other designs such as different electrical connectors. Some of them have dimming capability. The power in the space shuttle is 28 volts D.C. and the fluorescents run from 20 kilohertz inverters.

The fluorescent lamps at present in the shuttle are 4200 degrees K color temperature. They are fairly standard cool white phosphors. The new shuttle and subsequent lamp fixtures will probably be daylight 5600 K. Powers of the lamps vary from 6 to 33 watts and operating life is greater than 60% output at 6000 hours. One unique feature is that all the fluorescent tubes are sealed against mercury leakage. There was a fear in the early design that any mercury that might escape from a broken lamp would persist in the cabin for very long periods of time, so all the lamps have a teflon jacket which hermetically seals the lamp. You can actually break the lamp inside the jacket and it will still run for a period of time with just the teflon containing the gas. The lamps are also sealed inside the fixtures so there is a sort of double containment. But the price paid for this safety margin is that the efficiency of the interior fixtures is nowhere near what one would expect based on the 50-60 lumens per watt obtainable from ordinary fluorescent lamps. In addition to the main interior fixtures there are some special purpose fixtures such as the plant growth unit.

(Figure 2) This shows a typical small fixture used for lighting the instrument panel in front of the pilot. It is about 8 inches long and has 2 small T5 lamps, each at about 6 watts. You can see the teflon jacket. The lamp ends are totally encapsulated. The fresnel lenses for distributing the light onto the instrument panel are shown separated.

(Figure 3) One of the larger fixtures is shown here. The sunlight makes it difficult to see the light near the crewman's head.

(Figure 4) This is a typical light distribution pattern for the fixture just shown. There is a 3/8 inch thick book on the interior fixtures that just contains the specifications for the interior lights. This figure is from that spec book. That is part of the reason these fixtures are so expensive. The cost is largely for the paperwork rather than the hardware. The radiation pattern shown here has a peak of 15 foot candles or 150 lux at a 3 foot distance.

(Figure 5) This is the plant growth unit flown on one of the early missions.

(Figure 6) Briefly we will go through the exterior fixtures. The payload bay floodlight is a metal halide lamp. That lamp runs D.C. and is probably much higher power than one would ever consider for small area illumination. However, the important point is that the lamp dose can be changed for different spectral characteristics. Such a change probably does not require a total requalification of the lamp and fixture. In the same way, the phosphors in the fluorescent lamps can probably be changed without a total requalification.

(Figure 7) This shows the payload bay lamp itself. It has a double jacketed quartz body.

(Figure 8 & 9) These figures show payload bay fixtures that the lamp fits.

(Figure 10) One of the few incandescent lamps on the shuttle is the remote manipulator arm fixture. Its lamp is close to being an off-the-shelf item except there is an extra reinforcement on the filament so it can withstand the shock and vibration of launch.

There are a number of miscellaneous lights that have been used in space. The man maneuvering unit has small running lights. The astronauts use flashlights in some cases. There was even a 6W miniature xenon short arc lamp on the Mars Viking lander to simulate sunlight for a biology experiment.

This has been a quick description of some of the present space qualified sources. It is still undecided what lighting will go on the space station. (Figure 11) However, the interior illuminators will probably be fluorescent. There will probably be some miscellaneous incandescents on the interior. On the exterior, because of efficiency considerations, the lighting will probably be metal halide or alkali metal arc lamps. Of course there will be a number of specialized beacons and running lights.

Questions

(Alberts) What are the possibilities of using fiber optics to distribute the light with these sources?

(Schuda) Most of these sources are not ideally suited for fiber optic illumination. For that type of system one usually uses a high brightness source like a xenon short arc. However, there have been designs for things like plant growth units where fiber optic bundles were proposed to distribute the light from a single fluorescent.

(Alberts) Are there external lights which are turned on all the time on the shuttle?

(Schuda) I don't believe they have the payload bay lights on except when needed.

- **Up to 24 different Assemblies.**
- **All have internal power supplies, some have dimming capability**
- **5600°K daylight phosphor for subsequent shuttle fixtures, 4200°K on existing shuttle hardware**
- **Input power from 6.6 to 33 W maximum**
- **Operating life, >60% output at 6000 hrs**
- **All fixtures sealed against mercury leakage**
- **Special purpose fixture-plant growth unit**

Fig. 1 Interior Shuttle light assemblies.

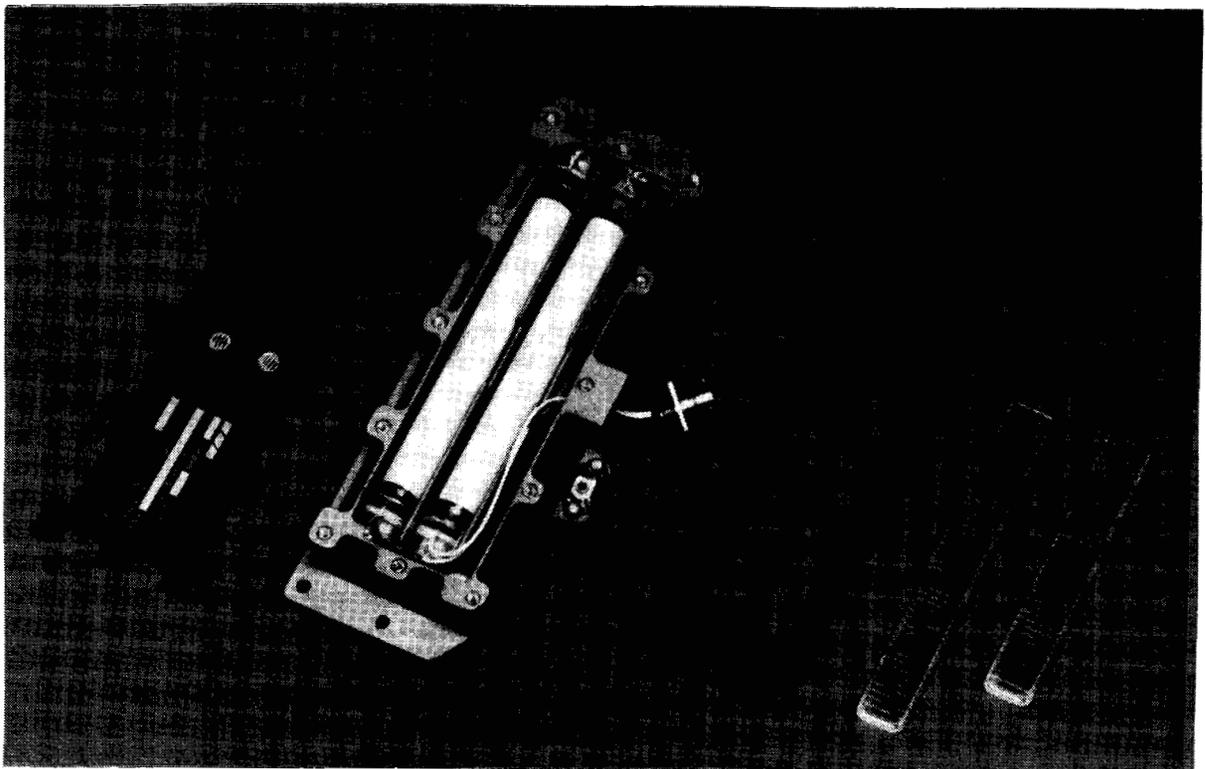


Fig. 2 Lighting fixture for instrument panel.

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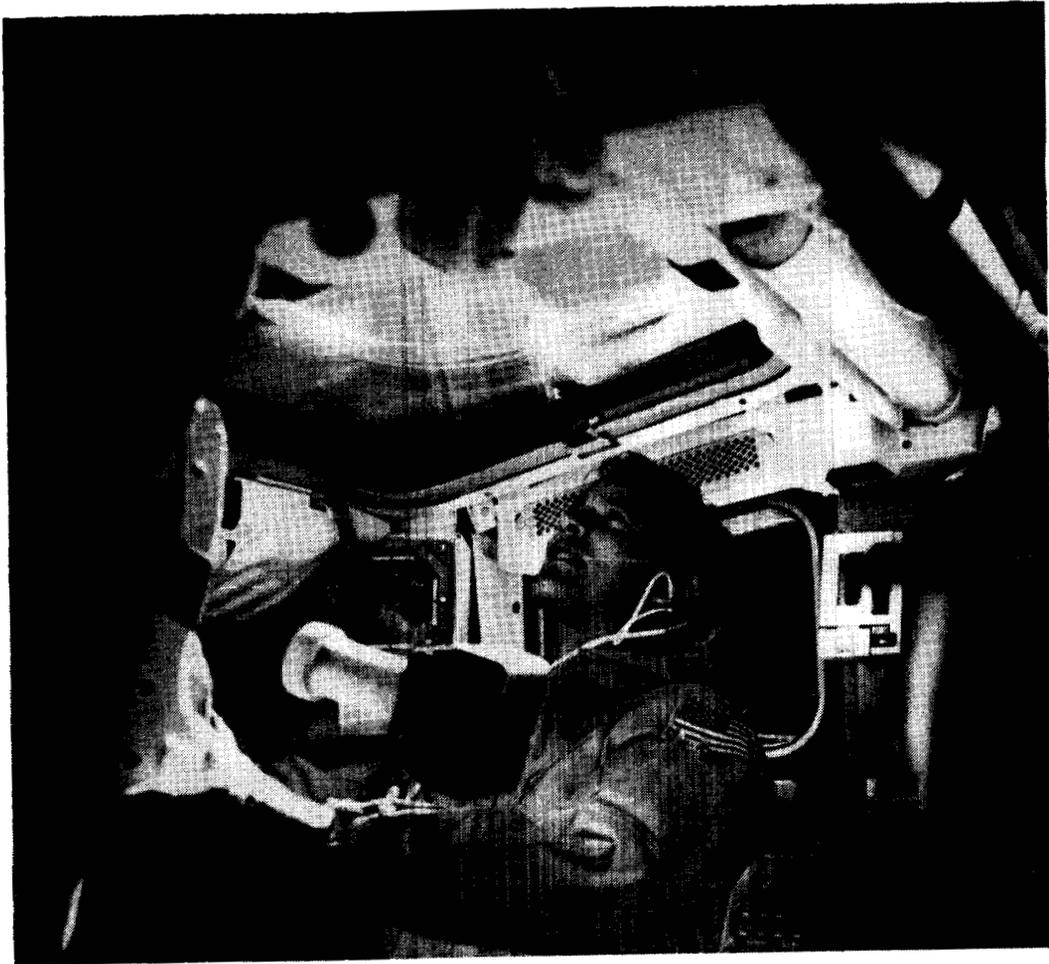


Fig. 3 Lighting fixture in place aboard Shuttle.

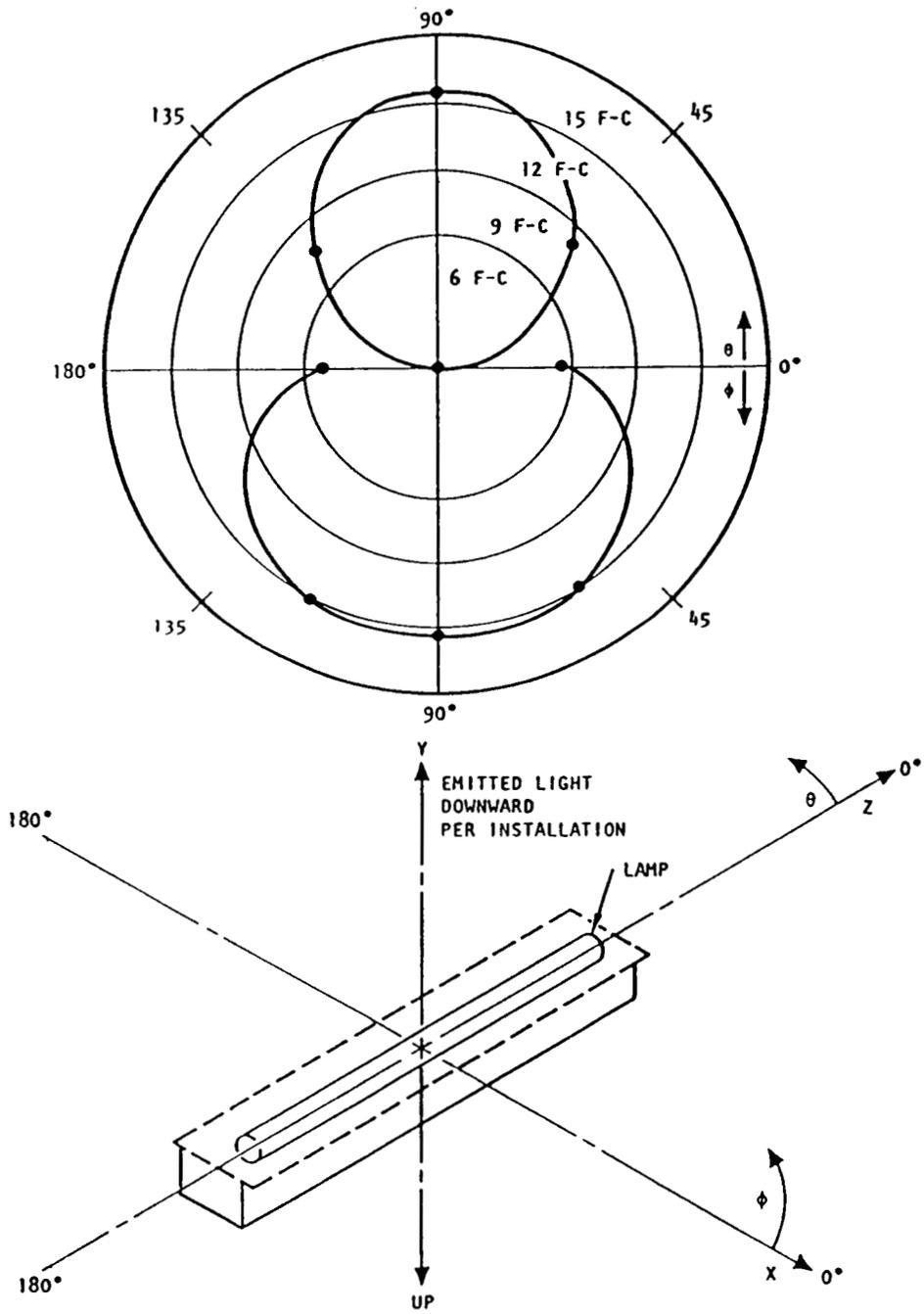


Fig. 4 Polar illuminance distribution.

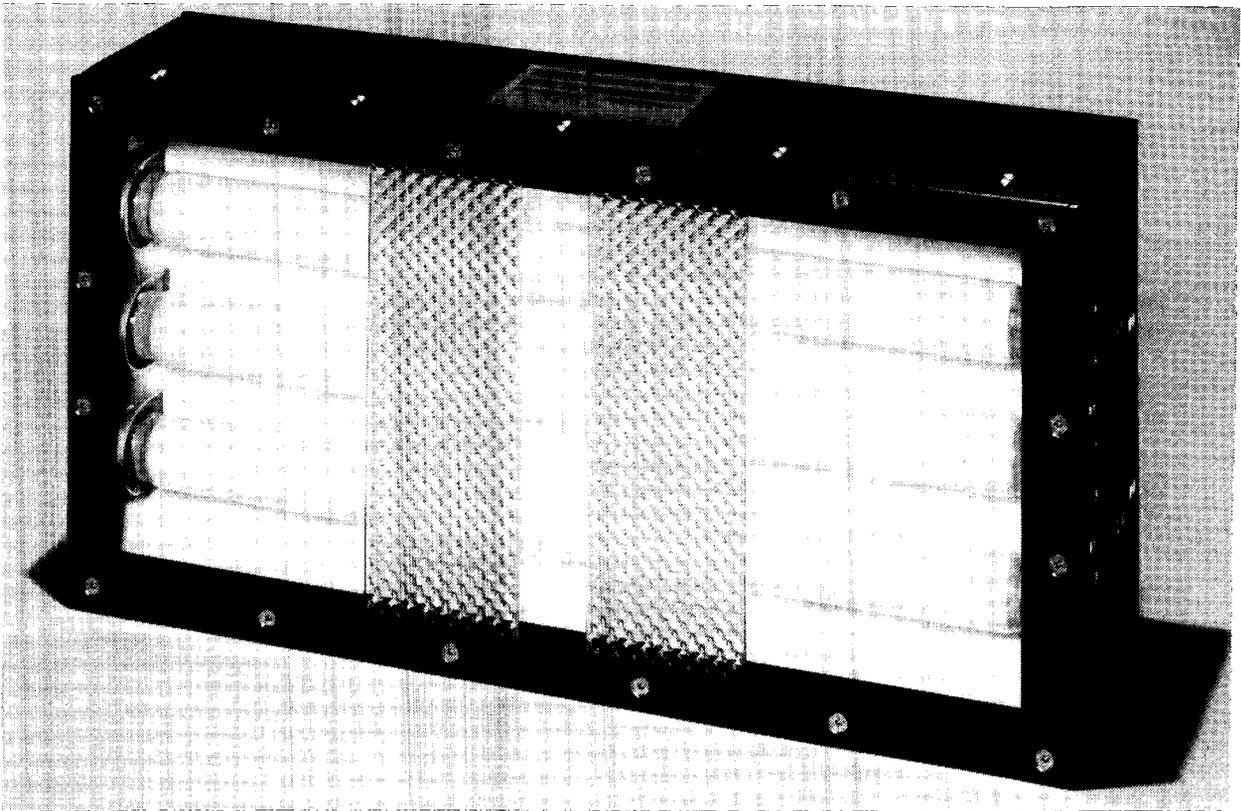


Fig. 5 Plant growth unit.

Payload Bay Floodlight

- **3200°K CCT**
- **Metal Halide DC discharge lamp**
- **175 W input power**
- **Dose can be changed to vary spectral characteristics**

Remote Manipulator Arm Floodlight

- **> 2800°K CCT Incandescent Lamp**
- **200 W input power**

Miscellaneous

- **MMU lights, running lights, "flashlights"**
- **6 W xenon short arc lamp used on Mars Viking Lander**

Fig. 6 Exterior Shuttle light assemblies.

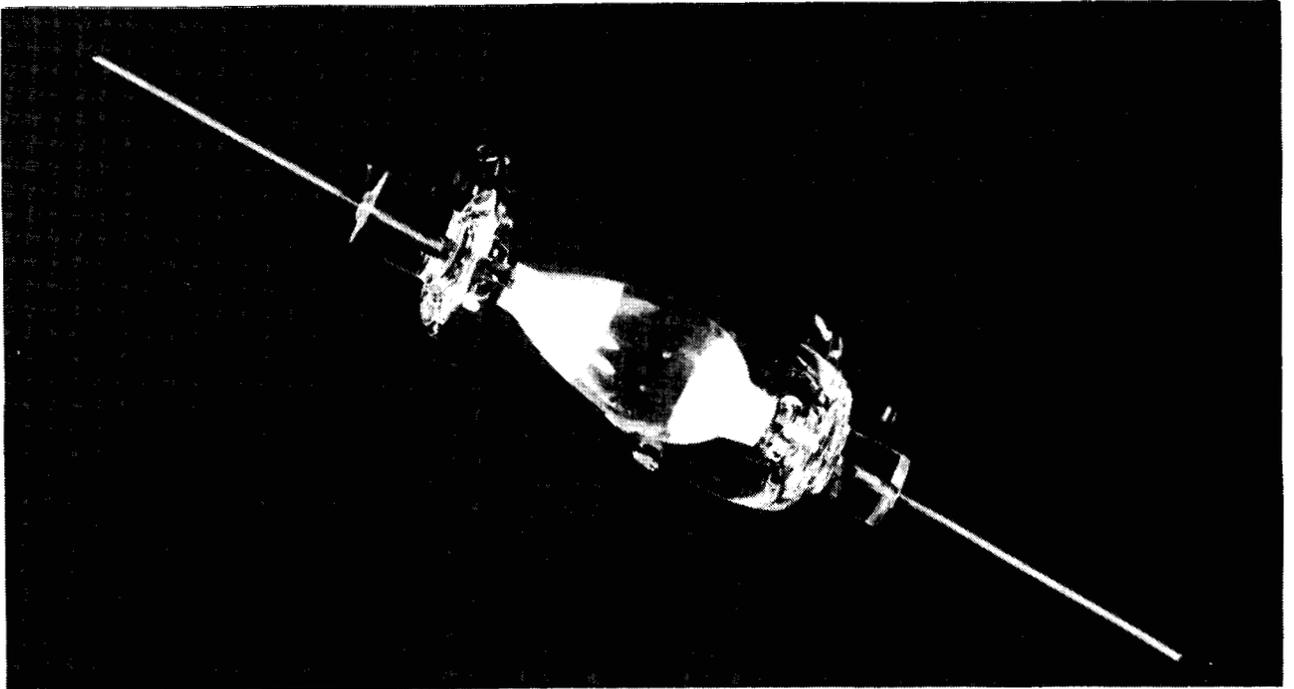


Fig. 7 Payload bay lamp.

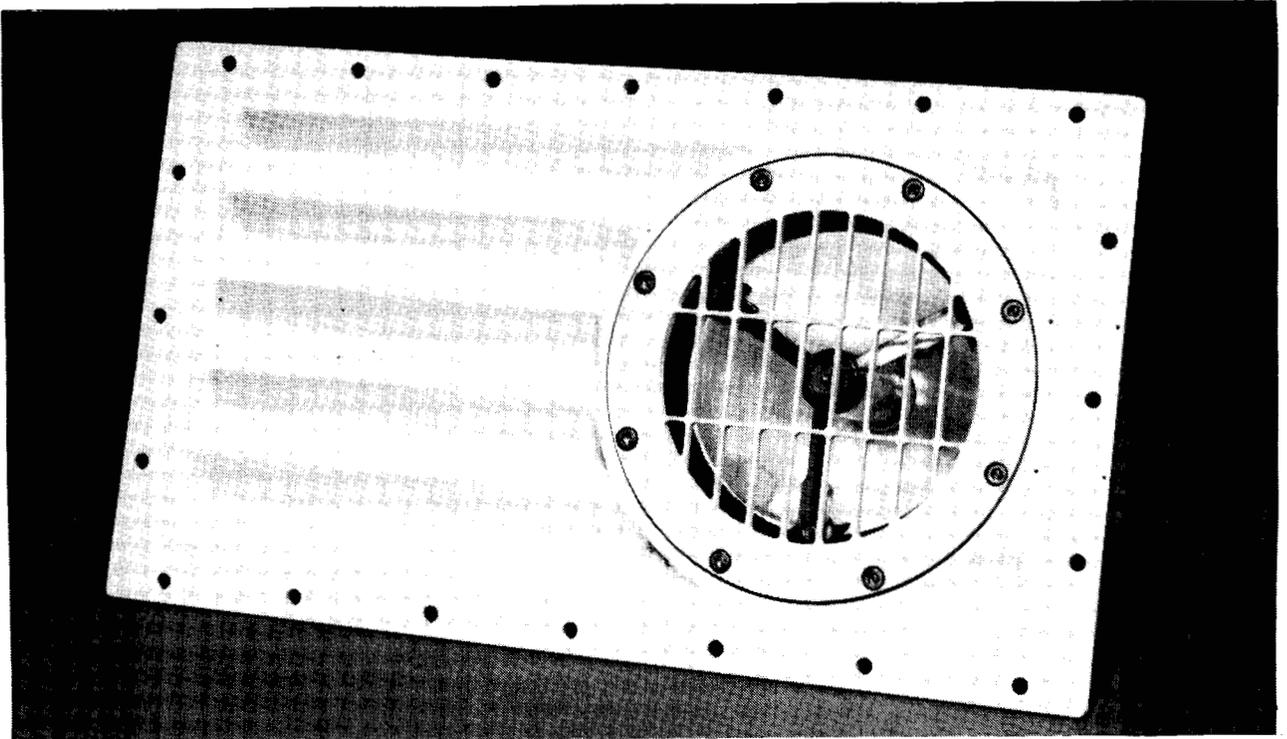


Fig. 8 Payload bay fixture --front view.

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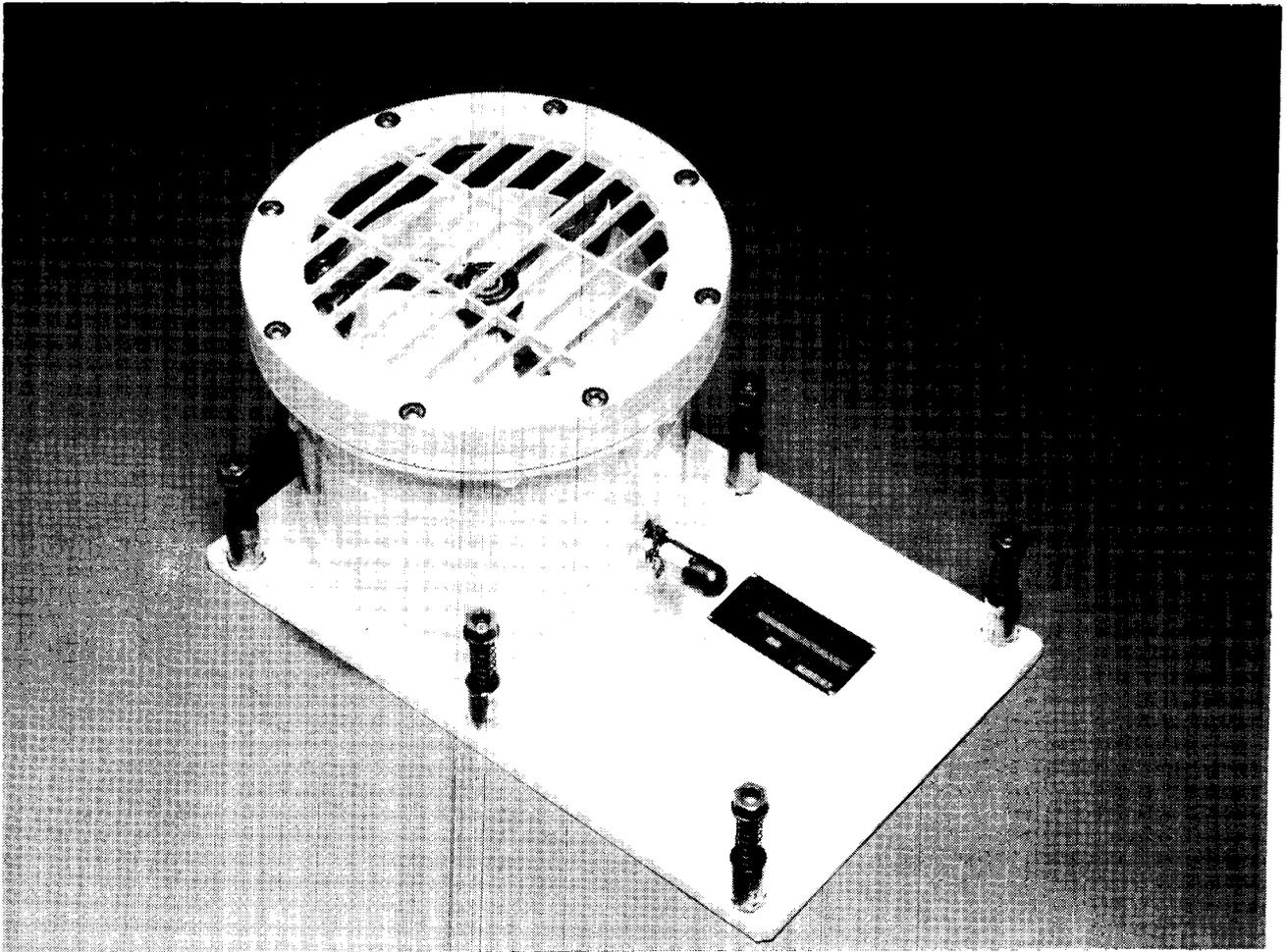


Fig. 9 Payload bay fixture--side view.

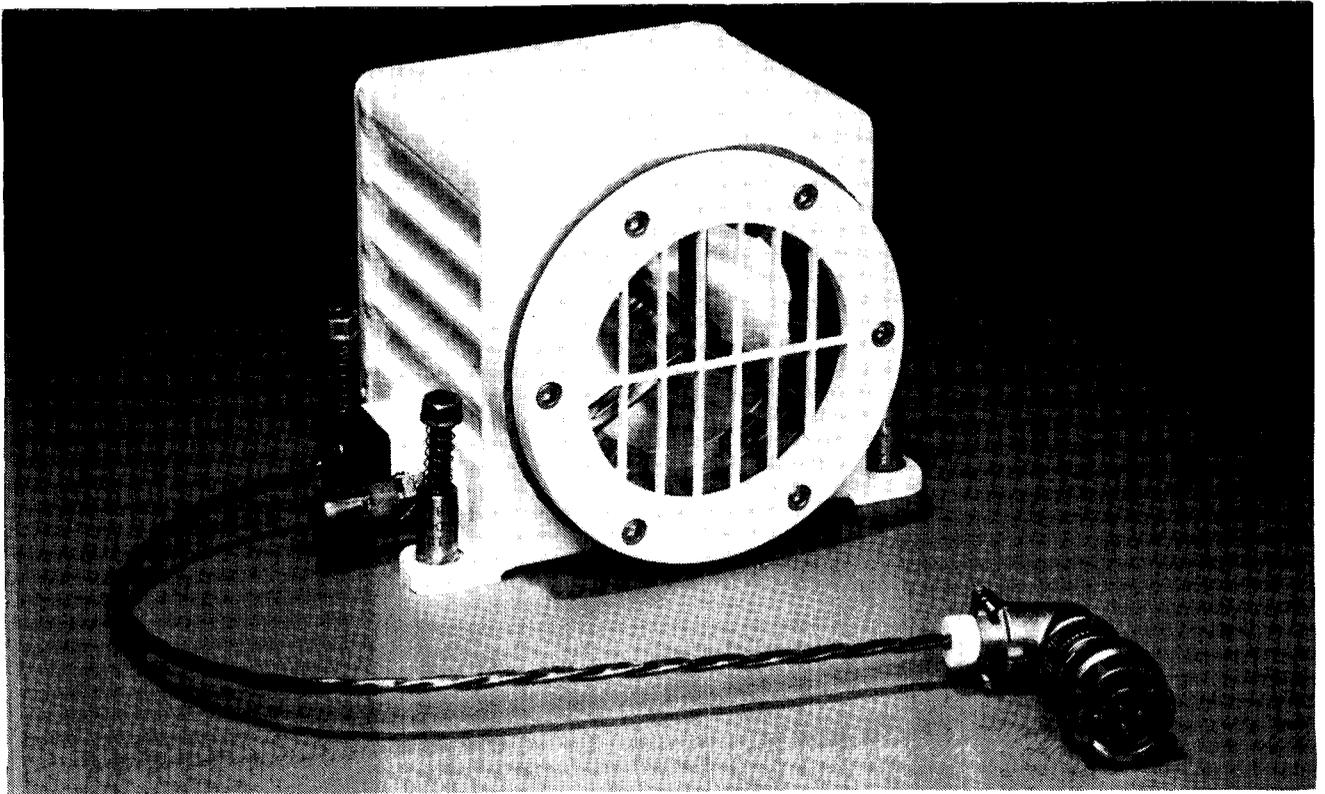


Fig. 10 Remote manipulator arm fixture.

- **fluorescents for interior general illumination and some task lighting**
- **incandescents for emergency lighting and some task lighting**
- **metal halides for large area external lighting**
- **miscellaneous running lights, strobes, camera lights etc.**

Fig. 11 Probable Space Station lighting.

THE MAMMALIAN CIRCADIAN SYSTEM AND THE ROLE OF ENVIRONMENTAL
ILLUMINATION

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ABSTRACT

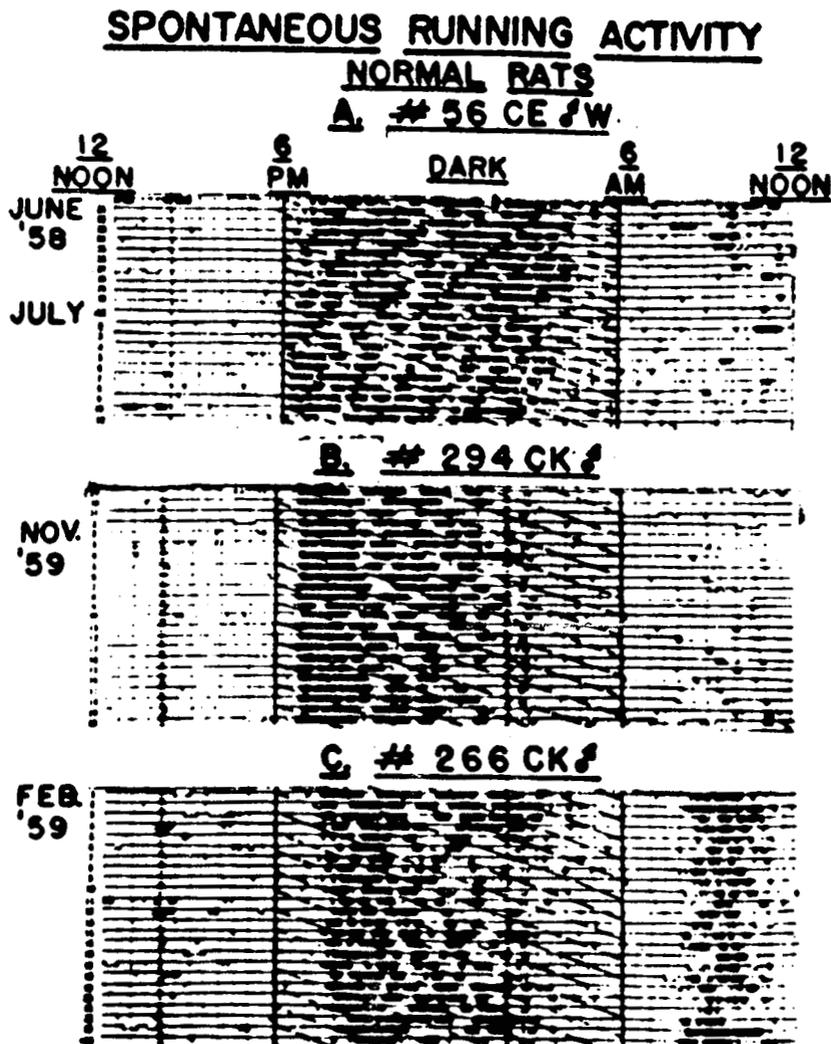
The term circadian is but one entry in the lexicon of chronobiology, a new science that deals with rhythms of life. A circadian rhythm is one with a period that approximates 24 hours. Through evolutionary time, environmental cycles have left their stamp on the basic biology of organisms. These cycles include the daily alternation of light and darkness; the yearly changes in day length; and the climatic and biotic cycles that these physical changes engender. There are corresponding rhythmic patterns in the behavior and physiology of animals. The temporal pattern of environmental light and darkness is clearly basic to the coordination of diverse circadian rhythms and "normal" function. For nearly a century rats have served as models in biomedical research but, until very recently, the question of what constitutes an optimum photic environment for laboratory rats has been neglected. The Laboratory Rat is a product of more than a century of selective breeding for vitality, docility, and fecundity in the laboratory environment. There, rat caging systems and husbandry practices have evolved that tend to favor the comfort and convenience of animal care personnel and investigators - not rats. Much methodological error has thus become institutionalized. Serious consideration of lighting requirements of animals in microgravity should help to rectify this situation. A brief review of some recent studies may help define the problem and suggest some broad guidelines for useful, humane, and practicable biomedical studies in space.

Most students of biology in the twentieth century were weaned on homeostasis, the concept that a set of regulatory mechanisms maintains constancy of the "internal milieu." Changes in biological function are thought to be counteracted or balanced to maintain an equilibrium or steady state. With an increasing ability to measure physiological changes over time, physiologists have been forced to broaden the concept of homeostasis to allow, not only for constancy of a variable function in its long-term mean, but also for recurrent variations around that mean which often appear in a rhythmic fashion. It is now recognized that these are not random variations but rather, they represent a vital, genetically determined part of the living system. There is an enormous variety of well documented rhythmic patterns in biological systems ranging in frequency from milliseconds in the firing of neurons to years in the oscillation of animal populations. In view of the ubiquity of such rhythms and the diversity of functions they serve, the rhythmic structure of biological systems must now be considered a principle, complementary to that of homeostasis.

There is a sub set of biological rhythms that are precisely synchronized with, and clearly represent dynamic adaptation to geophysical cycles. The latter result from the daily rotation of the earth under the sun and moon, the moon's monthly orbit of the earth, and the earth's annual circuit around the sun, its axis inclined with respect to the plane of the orbit. The corresponding endogenous biological rhythms have periods of roughly 24-hours, 12.5 hours, 29.5 days, and 12 months respectively.

Dr. Curt Richter pioneered the study of endogenous biological rhythms in mammals. Figure 1 is an illustration from one of Richter's publications (10). Here the entrained activity pattern of three rats is shown. Each rat enjoys access to a squirrel cage type revolving drum. A revolution of the drum causes the lateral deflection of a recording pen on a moving chart. Each horizontal line represents 24 hours in the life of a rat. The precise periodicity of daily rhythms is best displayed by this raster format plotting technique. The record for each day is placed below that of the preceding day so that events that occur at precisely the same time each day are lined up vertically on the page. The concentration of pen deflections during the hours of darkness illustrates the photophobic, nocturnal rat's disposition to concentrate his activity in the 12-hour dark portion of the day.

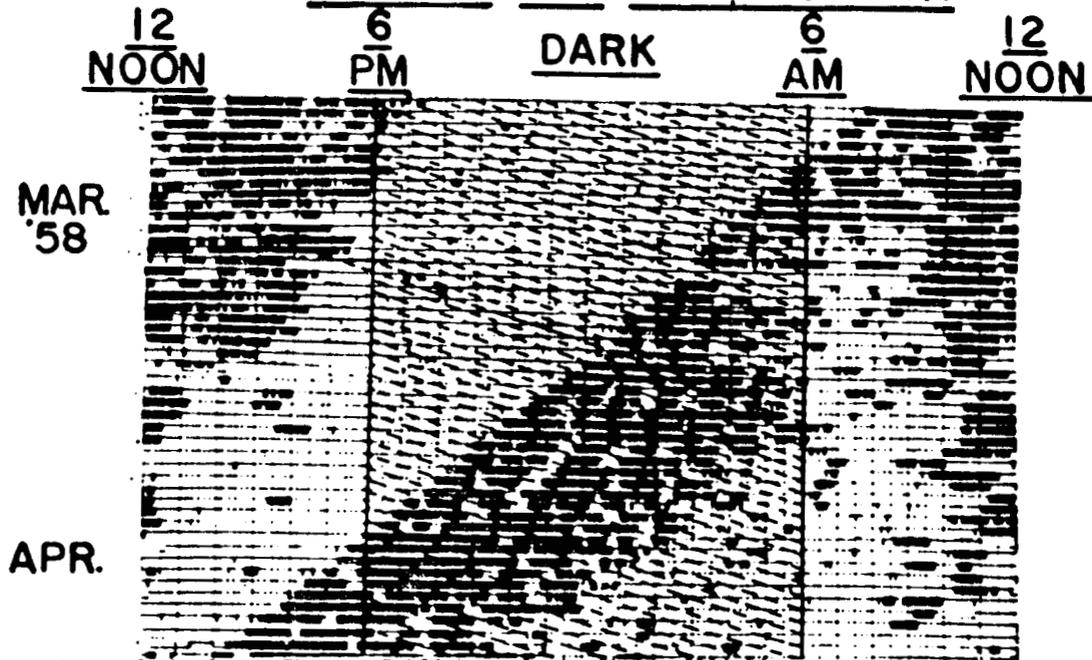
Figure 2 is another of Richter's illustrations. This time it's the free-running activity pattern of a blinded rat. Free-running because the activity pattern is driven by the rat's endogenous pace maker. It is not entrained by the pattern of environmental illumination. Note that the daily burst of activity begins about 10 minutes earlier each day. The animal is displaying a circadian period of about 23.8 hours. This is characteristic of what Richter called a 24- or nearly 24-hour rhythm. Since the period of a free-running rhythm only approximates that of the geophysical cycle with which it is normally synchronized, the Latin prefix, 'circa' was invoked by Franz Halberg in 1959 to characterize circadian rhythms (1), and then later adopted for the three other endogenous rhythms that correspond to cycles in the environment (circatidal, circalunar, and circannual rhythms).



Courtesy of Charles C Thomas, Publisher, Springfield, Illinois
FIGURE 1. Activity-distribution records for three normal rats. The laboratory was in total darkness from 6 P. M. to 6 A. M. (Richter, 1965)

SPONTANEOUS RUNNING ACTIVITY

BLINDED RAT # 243 CD♀WN



Courtesy of Charles C Thomas, Publisher, Springfield, Illinois

FIGURE 2. Activity-distribution record illustrating great accuracy of times of onsets of activity from day to day. This wild Norway (trapped in Baltimore) was blinded several weeks before the start of this record. (Richter, 1965)

Although circadian rhythms can free-run with their own periodicity, an animal's circadian system is normally entrained to a 24-hour period by environmental time cues (called zeitgebers), the most important of which is the light-dark cycle. Figure 3, from a publication by Moore-Ede (7), shows that after 8-hour phase shifts (first a phase delay and then a phase advance) in the timing of the light-dark cycle with respect to clock time, it takes several days for the rest-activity cycle of a squirrel monkey to resynchronize (entrain) to the new phase.

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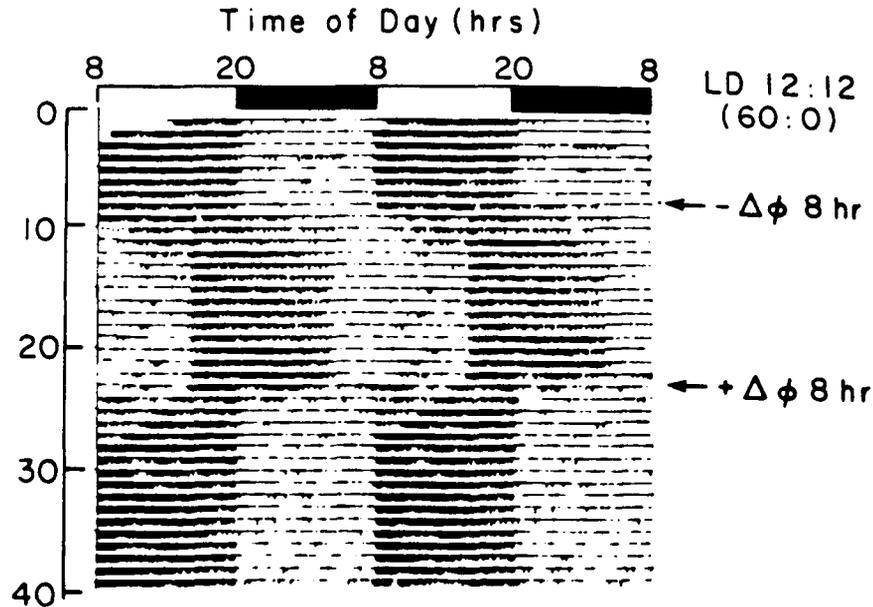


FIGURE 3. Reentrainment of activity rhythm of squirrel monkey after 8-h phase delay and 8-h phase advance of light-dark cycle. *Black bar*, time of darkness on days 1-9 and days 24-40. (Moore-Ede, 1986)

Experimental analysis of the entrainment of circadian pacemakers by light cycles is based on measurement of their phase response curves (PRC). If the pacemaker, otherwise freerunning in constant darkness, is acted upon by a brief light pulse, its phase is shifted relative to that of an unpulsed control. The sign (+ for advance, - for delay) and magnitude (in hours) of the phase shift is characteristic of the phase making oscillation's phase at which the light pulse exerts its influence. The convention is to describe the succession of pacemaker phases in terms of circadian time (ct); with the full cycle, a circadian day, lasting τ (tau) clock hours, one circadian hour = $\tau/24$. A specified circadian time (ct) is thus a pacemaker phase. ct 0 is taken as the pacemaker phase which coincides with dawn in the entrained steady state established by a cycle of 12 h light/12 h dark. The first half of the cycle (ct 0 to ct 12) is the subjective day; the second half (ct 12 to ct 24) is the subjective night. The curve relating this dependence of the sign and magnitude of phase shifts on the circadian time point at which the light pulse is imposed is called the phase response curve.

Figure 4 illustrates the phase response curve developed by Hoban and Sulzman (2) for the circadian pacemaker driving the drinking behavior of squirrel monkeys. The animals were maintained in constant darkness and exposed to one-hour pulses of light at various circadian time points. The general pattern of the squirrel monkey PRC is found in those of all other circadian pacemakers studied. Pulses of light falling during the subjective day elicit negligible phase shift responses; pulses falling

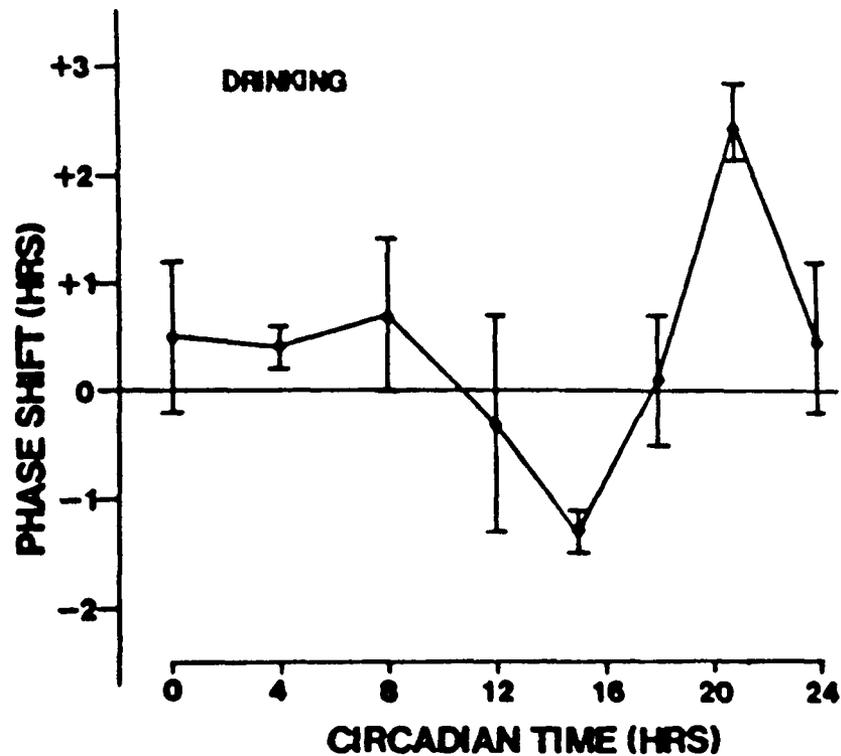


FIGURE 4. Phase-response curve to 1-h light pulses (600 lx) applied to squirrel monkeys maintained in constant darkness. Time scale is in circadian time where dawn would occur at *time 0*. Thus 0-12 h represent subjective day, and 12-24 h constitute subjective night. As is characteristic for phase-response curves to light, maximal phase delays occurred in early subjective night and maximal phase advances in late subjective night. (Hoban and Sulzman, 1985)

during the subjective night elicit large responses. Phase delays occur when light pulses are imposed during the early subjective night; phase advances result when the light pulses are imposed during the late subjective night.

These few examples illustrate specific instances, and some of the general properties of circadian rhythmicity. What is important about the circadian rhythm phenomenon is its pervasiveness. Virtually every parameter of animal physiology and behavior incorporates this temporal dimension, e.g., changes in body temperature and metabolic rate, the ebb and flow of circulating hormone levels, enzyme activity, susceptibility to disease, and the efficacy of nutrients and drugs.

In our laboratory, concern with environmental illumination stems from our interest in the mechanisms whereby physiological and behavioral variables in animals are adaptively harmonized with daily and seasonal changes in their environments. The defined cycles of temperature, of humidity, of light and darkness and of the biotic variables that these engender, somehow cause certain behaviors and physiological functions to be more advantageous for animals at one phase in the environment's total cycle, and less so at other phases. The extraterrestrial input that animates and imparts a temporal dimension to the biosphere is electromagnetic radiation from the sun. A portion of that radiant input is visible light. The biological variable that our studies have focused on - and whose synchronization with environmental light cycles is demonstrably adaptive, is rhythmic melatonin secretion from the mammalian pineal gland. Some of our experiences in the study of rhythmic pineal function illustrate important considerations in experimental manipulation of the photic environment.

Initial studies on the interaction of pineal function and the photic environment depended on inferences about melatonin synthesis and secretion that were based on measurements of pineal enzyme activity. Many of these studies compared melatonin synthesis between blind and sighted animals or intact animals maintained, for protracted periods of time in either continuous light or continuous total darkness. These studies established, some basic axioms of pineal function; that melatonin secretion tends to be increased when animals are exposed to darkness and diminished when they are exposed to light (12, 13).

With the demonstration that rhythmic melatonin production persists, phase-locked with the animal's circadian activity pattern, among animals kept in continuous darkness (8) and in blinded animals (9), rhythmic pineal function was established, both as a true circadian rhythm and one that is normally entrained with a fixed phase relationship to the day/night cycle.

Neither blindness, continuous light, continuous darkness, nor abrupt transitions from bright light to total darkness and vice versa are normal photic environments for normal rats. Laboratory rats have been selectively bred and cultured over many generations to endure, and indeed to thrive under such environmental conditions, it is very difficult to learn much, from studies performed under these conditions, about normal behavioral and physiological responses of rats in their natural environment. The most casual observation of rats in their natural habitat (i.e., wild *Ratus norvegicus* as domestic pests) reveals that their experience of environmental illumination is quite different from that of the laboratory rat (4).

The laboratory rat's wild ancestors evolved adaptations to a nocturnal fossorial way of life, which means that they tend to spend daylight hours in totally dark subterranean burrows, sleeping and passively evading diurnal predators. They spend nighttime hours, not in darkness, but rather in the dim light that prevails at night, foraging for food and actively evading nocturnal predators. We must conclude that the environmental illumination experienced by wild rats normally consists for the most part of total darkness by day (when they reside in their dark burrows), and very dim light (such as that provided by the moon and stars) by night.

We examined the relationship between ambient light intensity and melatonin excretion in laboratory rats (5). The animals were exposed to darkness, dim light (0.1-0.3 $\mu\text{W}/\text{cm}^2$), or bright light (45-100 $\mu\text{W}/\text{cm}^2$). Light was provided by sun light simulating Vita-Lite fluorescent tubes (Duro-Test Co., North Bergen N.J.). The dim light that was used approximated the light present outdoors between deep twilight and full moonlight, whereas the bright light approximated what is generally present in a laboratory environment. The animals were exposed during alternating 12-hour periods either to dim light and darkness, or to dim light and bright light. The daily pattern of melatonin secretion was assessed by measuring the melatonin content of urine samples collected over consecutive 12-hour periods at the time of transition from one state of environmental illumination to the other. As an index of the animal's activity pattern, water consumption was measured over the corresponding 12-hour intervals.

Rhythmic melatonin excretion among both groups of rats was entrained by alternating 12-hour periods of contrasting light intensities (figure 5). Maximum values occurred during the darker portion of the day, that is, total darkness for the group experiencing dim light and darkness, and dim light for the group experiencing dim light and bright light. The rhythmic pattern of water consumption was similarly entrained (table 1), with larger volumes consumed during the darker portion of the 24-hour day.

These results demonstrate that environmental light and darkness are mutually relative. They show that a given light intensity presented for a part of the 24-hour day (e.g., dim light 0.1-0.3 $\mu\text{W}/\text{cm}^2$) can be interpreted by the mammalian pineal as light or dark, depending on the light intensity available during the rest of the day. They further show that the daily rhythm in activity, as evidenced here by water consumption, remains phase-locked with the melatonin pattern, peak values occurring during the darker portion of the day. It should be pointed out that the light intensities reported here are not equivalent to the light intensities impinging on the animal's retina, nor even on the eye. They are merely reference values measured immediately inside the front of each cage. Light was provided by overhead luminaries. Within the cage, there was a considerable range of lower light intensities produced, for example, by the rat's shadow.

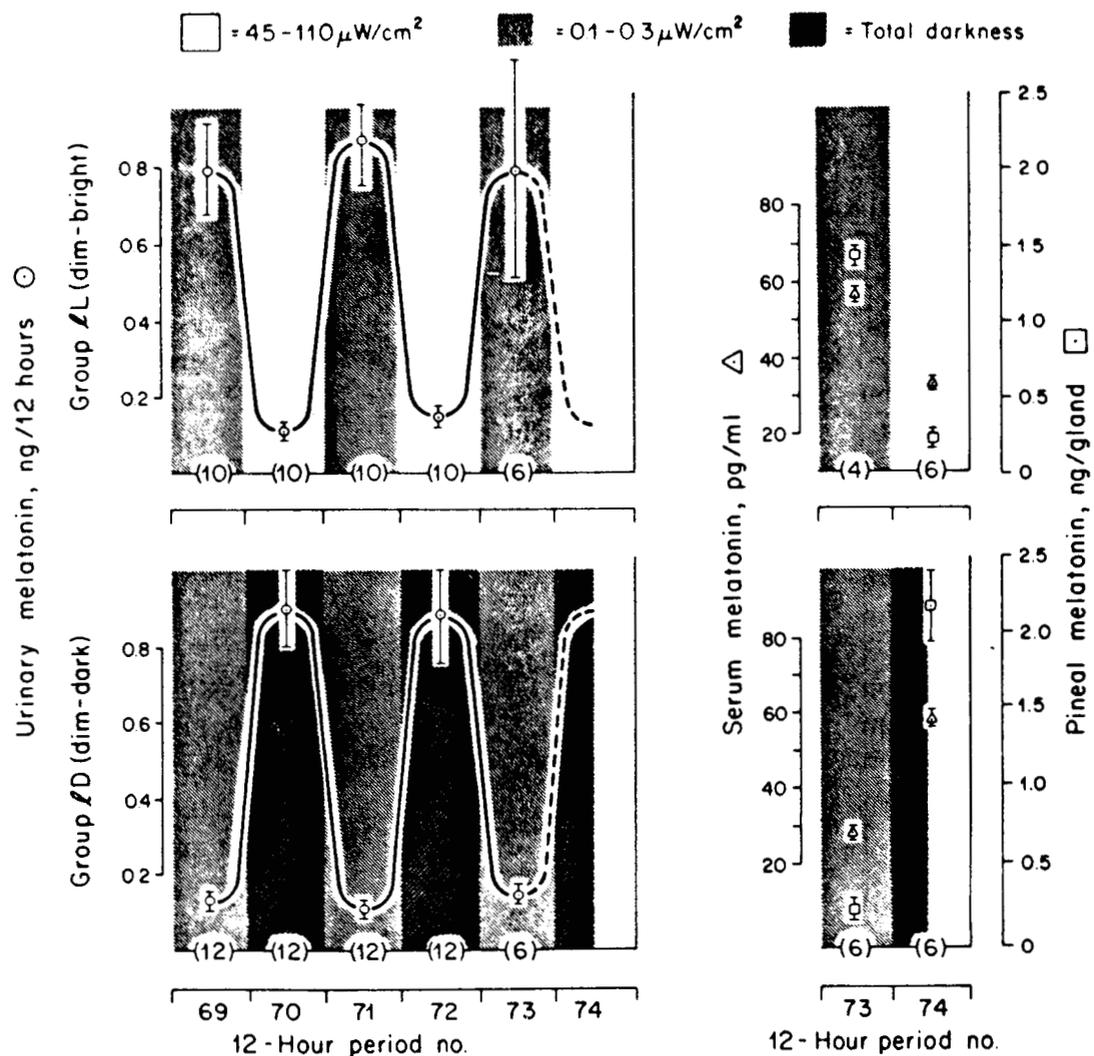


FIGURE 5. Urinary melatonin levels measured over consecutive 12-hour intervals from rats entrained to alternating 12-hour periods of either dim and bright light (top graph) or dim light and total darkness (bottom graph). Some animals from each group were killed in dim light at the midpoint of period 73 and the others either in bright light or total darkness at the midpoint of period 74, for measurement of pineal and serum melatonin levels (shown to the right). Dashed lines represent extrapolations of the rhythmic melatonin excretion patterns. Figures in parentheses indicate the number of animals in each group. Vertical bars show SEM (Lynch, *et al.* 5)

TABLE 1. Partial Record of Water Consumption Measured Over Consecutive 12-Hour Intervals

| Diurnal Lighting Period No. | Group: Dim/Dark | | Group: Dim/Bright | |
|-----------------------------|-----------------|------------------------|-------------------|------------------------|
| | Lighting | Water Consumption, ml. | Lighting | Water Consumption, ml. |
| 69 | dim | 4* | dim | 33 |
| 70 | dark | 28 | bright | 5 |
| 71 | dim | 6 | dim | 33 |
| 72 | dark | 32 | bright | 6 |

*Each value represents the mean of the volumes of water consumed by 12 rats. (Lynch, *et. al.* 5)

If among nocturnal, burrowing, photophobic animals like the rat, melatonin secretion were controlled strictly by environmental illumination, occurring during the daily dark period and invariably suppressed by light, and if rats in their natural environment spent the daylight hours in dark burrows and nighttime under moon and starlight actively foraging for food and actively evading predators, then melatonin secretion might be expected to occur during the daytime and to cease at night, that is, out of phase with the animal's activity pattern. In the Spartan environment of laboratory cages, lacking dark burrows, melatonin secretion and activity are synchronous rhythmic phenomena, entrained by the daily lighting schedule or free-running in continuous darkness. To determine whether the daily rhythms in activity and/or melatonin secretion might be dissociated from the daily dark period in a simulated natural environment, we monitored both rhythms among rats housed in cages equipped with dark burrows (6).

Normal laboratory animal room daytime illumination ($66 \mu\text{W}/\text{cm}^2$) was chosen to represent the daylight condition. In these studies, 12-hour daylight periods alternated, not with darkness, but with 12-hour periods of nightlight corresponding in intensity to that provided naturally on the earth's surface by phases of the moon ($0.030 - 0.066 \mu\text{W}/\text{cm}^2$). Light in the exposed portion of the cage was provided by sunlight simulating Vita-Lite fluorescent tubes. Light intensity within the burrows was less than $0.013 \mu\text{W}/\text{cm}^2$ at all times.

To assess locomotor activity of rats housed singly in two-compartment metabolism cages, an aluminum treadle attached to a mercury switch was mounted in the tunnel that connected the dark burrow to the unshielded portion of the cage. The switch was closed when the animal traversed the tunnel from the lighted compartment into the darkened burrow, and opened when the rat went from the burrow to the lighted compartment. The switch energized one channel of an Esterline Angus event recorder deflecting a pen when the switch was closed. Thus, a detailed record was obtained of the amount of time the rat spent in either compartment and the frequency of its transits (figure 6).

To assess the pattern of melatonin secretion, consecutive 12-hour urine samples were collected from each of three rats housed individually in two-compartment metabolism cages. The melatonin content of each urine sample was measured by radioimmunoassay (Table 2).

These studies show that rats maintained under diurnal illumination in cages modified to provide continuous access to dark burrows, would remain almost constantly in their dark burrows during the most brightly lit portion of the day. They emerge from their burrow either when, or shortly after, ambient light intensity drops to a nighttime level. Locomotor activity is confined almost exclusively to the night light period. Rhythmic melatonin secretion occurs in phase with the rat's activity, and peaks during the nightlight period.

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SELF-SELECTED EXPOSURE TO LIGHT

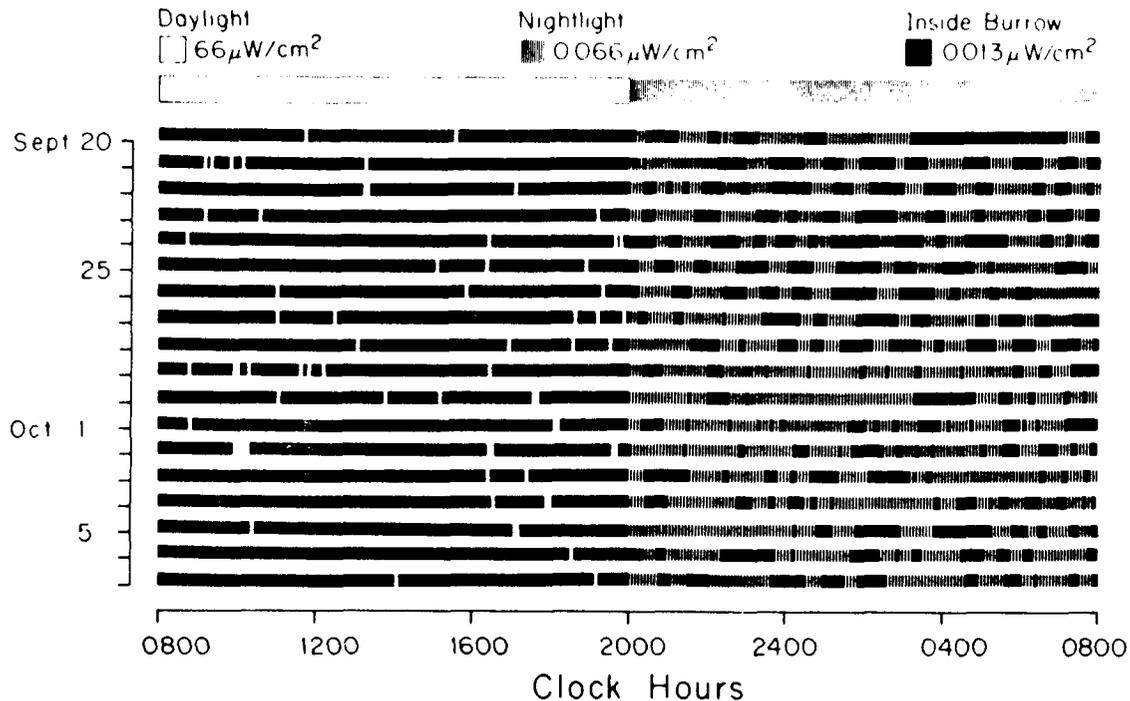


FIGURE 6. The self-selected exposure to light over a period of 18 days of a single rat in a cage equipped with a dark burrow. Horizontal black bars indicate time spent in the burrow each day. Interruptions in the black bars represent the frequency and duration of emergences of the animal from the burrow into the prevailing light intensity, indicated by the bar at the top of the chart. (Lynch, *et al.* 6)

TABLE 2. Melatonin Excretion (ng/12 hours)

| Rats in Standard Cages | | Rats in Cages Equipped with Dark Burrows* | |
|------------------------------|------------------|---|------------------|
| Rat #1 | | Rat #4 | |
| days | nights | days | nights |
| 2.2 | 2.5 | 1.7 | 3.1 |
| 1.7 | 4.4 | 1.0 | 3.0 |
| 1.9 | 3.8 | 1.2 | 2.6 |
| <u>1.9 ± 0.1^b</u> | <u>3.6 ± 0.6</u> | <u>1.3 ± 0.2</u> | <u>2.9 ± 0.2</u> |
| Rat #2 | | Rat #5 | |
| 1.1 | 3.2 | 1.0 | 2.6 |
| 1.5 | 2.8 | 1.0 | 2.3 |
| 2.2 | 2.2 | 0.2 | 1.9 |
| <u>1.6 ± 0.3</u> | <u>2.7 ± 0.3</u> | <u>0.7 ± 0.3</u> | <u>2.3 ± 0.2</u> |
| Rat #3 | | Rat #6 | |
| 1.0 | 2.7 | 1.3 | 4.3 |
| 0.9 | 3.1 | 1.3 | 4.0 |
| 1.7 | 2.5 | 0.7 | 2.6 |
| <u>1.2 ± 0.3</u> | <u>2.8 ± 0.2</u> | <u>1.1 ± 0.2</u> | <u>3.6 ± 0.5</u> |

*Light intensity inside burrows was less than 0.013 μW/cm². (Lynch, *et. al.* 6)

^bMean ± SEM

At this point, all of our studies had involved Sprague-Dawley albino rats. Since ocular pigmentation diminishes the proportion of incident light passing through the eyes and reaching retinal photoreceptive cells, we reasoned that the use of an albino animal model for our studies on light effects might lead to erroneous conclusions about what light actually does (e.g., in nonalbino animals and people), and might generate experimental artifacts that would preclude detection of other, more subtle influences.

Some preliminary studies with Brown Norway rats, showed that they indeed found the daylight intensities used in these studies much less aversive. Because these animals were quite expensive and not continuously available, we chose as our test animal pigmented Long Evans rats. Like the Brown Norway, Long-Evans hooded rats have been shown to be behaviorally much more tolerant of ambient illumination than Sprague-Dawley albino rats in both operant and spacial preference tests.

Further, to expedite this phase of our study, we adopted a popular experimental paradigm to assess the relative sensitivities of pineal function in different types of animals to light, namely, the acute suppression of melatonin levels caused by exposing animals to light during the dark phase of the daily light/dark cycle.

Figure 7 shows the pattern of nocturnal elevation in pineal melatonin content observed in both Sprague-Dawley albinos and Long-Evans pigmented rats.

Groups of Sprague-Dawley albino and Long-Evans pigmented rats were simultaneously exposed during the dark phase of the light/dark cycle to various intensities of diffuse, overhead illumination for 30 minutes. Pineal glands and trunk blood samples were then collected and assayed for melatonin content.

As shown in figure 8, albino rats exposed to irradiances of 0.110 uW/cm^2 or less had pineal melatonin levels that were not significantly different from those of unexposed animals. Higher irradiances significantly reduced melatonin levels. By contrast, as shown in figure 9, as little as 0.022 uW/cm^2 significantly reduced pineal melatonin levels in pigmented rats.

Serum melatonin levels measured in the same animals (table 3), confirm the surprisingly greater sensitivity of the pigmented rats to suppression of melatonin by acute exposure to light at night. As pointed out by Williams (11), the explanation of this anomaly may reside in the fact that the albino animals used in this study were obtained from a commercial source and their retina may well have been damaged by prior exposure to "normal" laboratory illumination.

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MELATONIN LEVELS IN
LONG-EVANS AND SPRAGUE-DAWLEY RATS

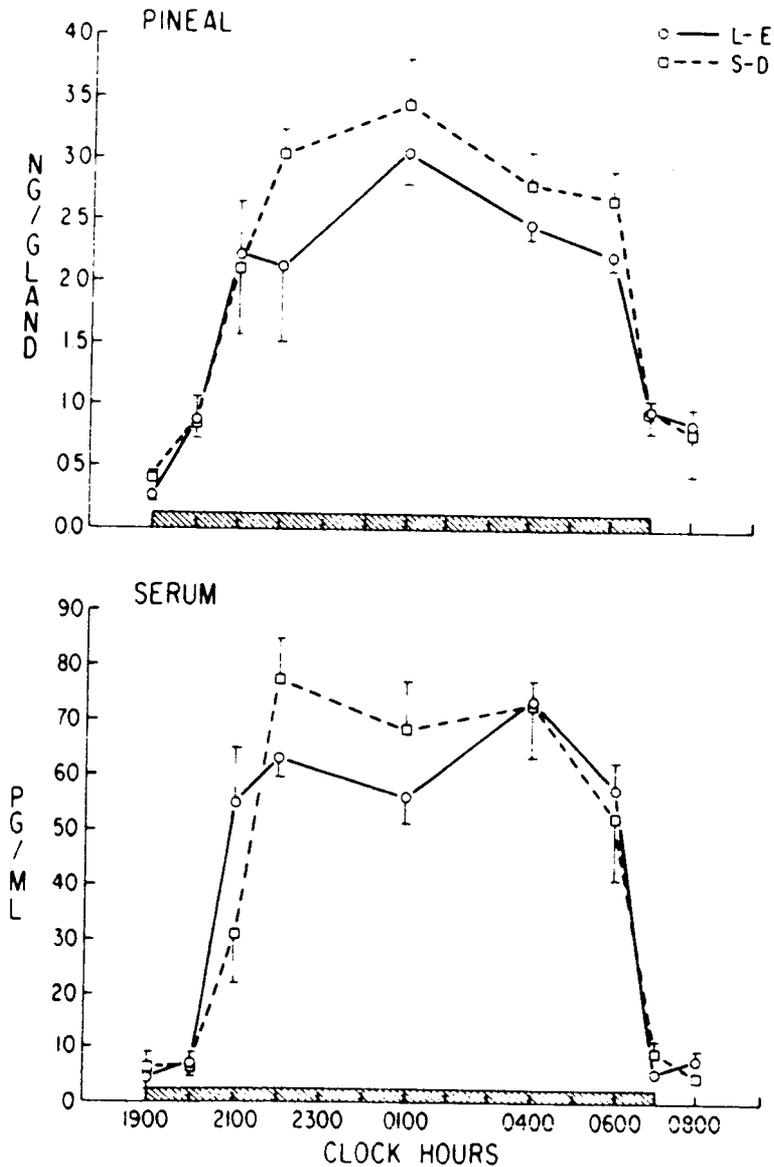


Fig. 7. Changes in pineal (upper panel) and serum (lower panel) melatonin levels throughout a dark cycle. Male Sprague-Dawley and Long-Evans rats were acclimated for three weeks to a lighting schedule of LD, 12 : 12 ($L = 200 \mu W/cm^2$; $D = 0.002 \mu W/cm^2$, red light $< 650 \text{ nm}$). Each point is the mean value from 3-6 animals. Vertical bars represent one standard error of the mean. Rats were killed in the light at 7 p.m., immediately before the onset of darkness, and in the dark at 7 a.m., immediately before the onset of light. Shaded bars represent the period of darkness

Lynch and Deng, J. Neural Transmission. 21 [Suppl]: 461-473, 1986.

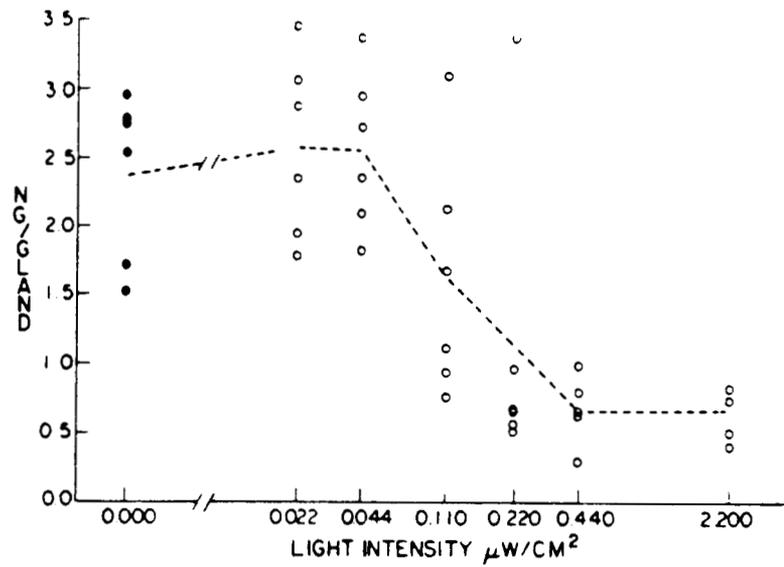


FIGURE 8 • Pineal melatonin contents of Sprague-Dawley rats exposed to various light intensities. Animals were killed in the dark 6½ or 9 hr after the onset of darkness (●) or after a 30-min exposure to one of 6 different light intensities (○). The broken line connects the mean value for each group. ● represents the mean ± SEM pineal melatonin content of 6 rats killed in the dark 9 hr after the onset of darkness. (Lynch, *et al.* 3)

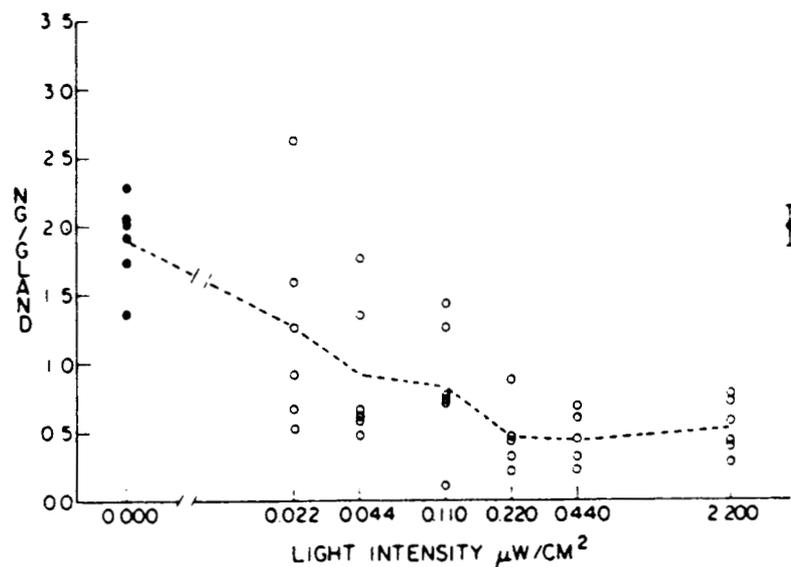


FIGURE 9. Pineal melatonin content of Long-Evans hooded rats exposed to various light intensities. For lighting conditions and key to symbols, see legend to FIGURE 8. (Lynch *et al.* 3)

TABLE 3. Serum Melatonin Concentration

| Light Intensity ($\mu\text{W}/\text{cm}^2$) | Sprague-Dawley Albino Rats (pg/ml) | Long-Evans Hooded Rats (pg/ml) |
|---|------------------------------------|--------------------------------|
| 0.000 | 67 \pm 7* | 52 \pm 7 |
| 0.022 | 62 \pm 6 | 22 \pm 7 |
| 0.044 | 66 \pm 4 | 20 \pm 7 |
| 0.110 | 27 \pm 11 | 6 \pm 1 |
| 0.220 | 26 \pm 14 | 8 \pm 1 |
| 0.440 | 12 \pm 2 | 7 \pm 1 |
| 2.200 | 9 \pm 1 | 6 \pm 1 |

*Mean \pm SEM (Lynch *et. al.* 3)

These few studies illustrate some of the complexity in the relationship between environmental illumination and behavioral and physiological rhythms. In addition to daily changes in light and corresponding changes in physiology and behavior, there are correlations between the annual variations in photoperiod length and circannual changes in physiology and behavior that have also been demonstrated. Many of these topics discussed elsewhere in this symposium.

Two aspects of the classic approach to animal rhythm study have failed to keep pace with the development of analytical methodology: the definition and measurement of relevant light, and the assessment of motor activity as it relates to an animal's exposure to light.

Light, defined as the visible fraction of that mysterious essence called radiant energy, is easily measured and manipulated. Visible, however, means different things to different animals, and many of the measurements of natural and artificial light that appear in the literature are not very useful in comparative studies. They all too frequently use psychometric units that relate only to humans. The most common of these human related units are the lux, phot, and foot-candle for illuminance, and the lambert and candela for luminance.

Luminance and illuminance are the psychometric equivalents of the physical parameters, radiance and irradiance. Integral to each of the psychometric measures is the spectral sensitivity of the human eye, usually for photopic (i.e., bright-light) vision. The basic problem is that psychometric units contain no information about the spectral distribution of the light measured, so that even if the sensitivity of the animal is known, the visual effectiveness of the light can not be assessed. We, and most other laboratories have adopted the practice of describing experimental illuminations by identifying the light source with its characteristic emission spectrum, and reporting incident light in radiometric terms. Thus, irradiance, usually expressed in W/cm^2 , is the amount of radiant flux received by a unit of surface area. This is a reproducible, physical value independent of its biological meaning.

There is an abundant literature on global illumination that provides the latest scientific information available on natural illumination. For clear days and clear nights, the illumination falling on a fully exposed horizontal plane at any point on earth, at any day of the year, at any hour of the day or night, CAN be found simply and quickly. Such global illumination estimates, however, simply do not describe the environment or photic experience of animals. In gross terms, animals are classified as either diurnal, nocturnal or crepuscular. These terms roughly describe an animal's behavioral disposition to experience global illumination. Their microhabitat and their ethological habit profoundly affects an animal's exposure to light.

Obviously the photic environment of choice for studying the effects of a novel environmental variable (e.g., weightlessness), would be the photic environment in which the animal normally lives and in which it has evolved the requisite sensory capacities and behavioral responses to optimally function. Such field studies are currently impracticable. However, a rational design of the experimental set up must include consideration of an animal's ecological and ethological roots.

CONCLUSIONS AND RECOMMENDATIONS

Since we are dealing with an urgent problem that has not been adequately investigated on earth, at one gravity, any proposals for properties of a photic environment in microgravity must be advanced very tentatively. However, on the basis of what we have learned, some very broad generalizations can be suggested; subject to relatively quick and inexpensive "ground testing" before they are implemented.

For studies involving laboratory rats

A - Properties of light

1) Spectral composition: Because what the animal "sees" is determined both by source parameters and reflective properties of the cage material and the animal, luminaries should provide light similar to natural sky light and the interior of the cage should be nearly flat black.

2) Intensity: The intensity should be sufficiently low that the retinae of animals are not damaged by exposure to it. Something on the order of 10 lux (= one foot-candle = 4.4 uW/cm_2) of daylight simulating white light would probably be adequate for the "day-time" condition (see Williams' review for specifics on this issue). The "night-time" condition might be represented by very dim ($< 0.002 \text{ uW/cm}_2$), very red ($> 650 \text{ nm}$) light (see Lynch and Deng, J. Neural Trans., Suppl. 21, 461-473, 1985). Spectral properties of the light and its irradiance should be recorded.

3) Quality: Irradiance should be presented from a diffuse source. The lens of the rat's eye focuses images on the retina. Apart from the injury that a focused bright source might inflict, on the retina, nothing is known about what other effects such exposure may have. The irradiance should be presented unidirectionally, i.e., from one side or the other of the cage. No effort should be made to immerse the animal in uniform illumination. If it were possible, it would be radically unnatural and counterproductive.

4) Temporal pattern: As outlined above, exposure to alternating light intensities within an appropriate range and with a period of near 24 hours is essential to maintain entrainment of endogenous rhythms and prevent individualization of circadian patterns in different animals. A skeleton photoperiod consisting of two brief pulses (e.g., 1-hr) of "day-light" to mark dawn and dusk would accomplish entrainment.

5) Darkness: Total darkness or near total darkness should be available at the option of the animal and the cage should be so designed to make this possible.

B - Caging

1) Size: Cages should be as large as possible, consistent with accommodating a sufficient number animals for statistical assessment of results and allowing some spacial preference options to the rats.

2) Containment of wastes: Air sweeps provide up-down orientation cues in the absence of gravity.

3) Location of dim, diffuse light sources: Luminaries could be mounted "overhead" at the end(s) of the cage space (one illuminated during the day; the other during the night). In the absence of a truly dark retreat, this arrangement would allow the animal to participate in determining his own exposure to light, simply by changing his head-tail orientation in the cage. With the Day-light source located at one end of the cage and the night light source at the opposite end. Preferred orientation could be monitored during experimental days and nights.

4) Food and water: Provisions should be made available at each end of the cage and consumption of food and water from the two sources monitored separately.

C - Animals

1) Species: Clearly the laboratory rat (a derivative of *Ratus norvegicus*) is the animal of choice for initial studies of animal physiology in a microgravity environment. He is a rugged adaptable animal, vastly more tolerant of stress than his wild relatives and most other mammals.

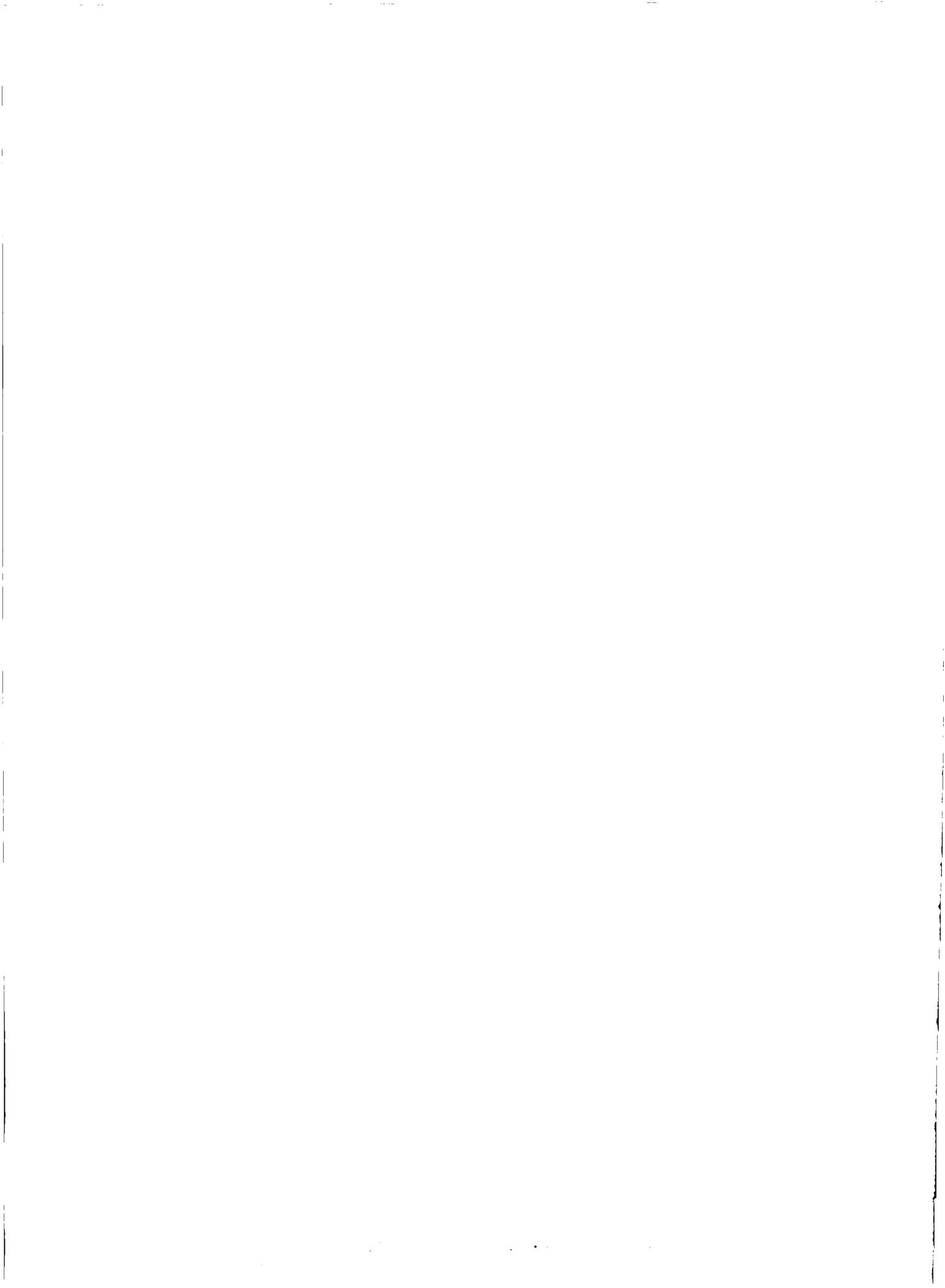
2) Pigmentation: A pigmented strain of laboratory rats should be chosen because they have the capacity to physiologically accommodate to changes in light intensity and would therefore tend to be more behaviorally and physiologically tolerant of any failure to accurately optimize the lighting environment. The Brown Norway strain would probably be the best. It is a more recently derived pigmented strain, better defined, and less heterogeneous in its genetic constitution than most other pigmented stocks available.

For studies involving non-human primates

The general principles concerning the entrainment of circadian rhythmicity are the same for all mammalian species studied to date. Specific recommendations for management of the photic environment of primates in microgravity should be left to people more experienced in working with those animals. Because of the phylogenetic propinquity of diurnal, experimental primates and man, a measure of anthropomorphism in the design of their accommodations might be justified.

References

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EFFECTS OF ENVIRONMENTAL LIGHTING ON RODENT CIRCADIAN RHYTHMS

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INTRODUCTION

Circadian rhythmicity of biological processes is the overt expression of an endogenous timing system. The rhythms are normally coordinated (entrained) to the 24-hr period by daily environmental time cues, with the daily light-dark cycle being the most potent entraining stimulus. Entrainment ensures a state of internal temporal order whereby the rhythms are expressed in proper relationship to each other and to the 24-hr day. This helps optimize the economy of biological systems and better prepares organisms to foresee and to cope with predictable alterations in the environment.

Along with its entrainment function, the daily light-dark cycle also acts through the circadian timing system to provide photoperiodic species with information about daylength (photoperiod). This photoperiodic function of light enables animals to anticipate seasonal changes and to vary biological responses, such as reproductive function, accordingly. Thus, the circadian timing system adjusts physiological processes to accomodate predictable changes in the environment that result from both daily and annual solar cycles.

In most rodents, the neural mechanisms necessary for both entrainment and daylength measurement are immature in the fetus. At this stage of development, the mother acts as a transducer between the environment and her young, communicating information about ambient lighting to the developing animal. Maternal-fetal communication of circadian phase ensures that as the

mechanisms necessary for the expression of circadian rhythms mature, developing rhythms are expressed in proper temporal relationship to each other and to the 24-hr day. Maternal-fetal communication of daylength, along with postnatal perception of photoperiod, allows the developing animal to determine which way the daylength is changing and to appropriately modify the rate of reproductive maturation. These maternally mediated forms of fetal light perception optimally prepare the developing mammal for its entry into the outside world.

THE CIRCADIAN TIMING SYSTEM OF ADULT MAMMALS

Conceptually, the circadian timing system can be divided into three major components (1; Fig. 1); a circadian pacemaker (biological clock) that drives the system, input pathways that entrain the pacemaker to the 24-hr day and output pathways that couple the pacemaker to other neural structures for the overt expression of circadian rhythms. Based on extensive studies in rodents, the suprachiasmatic nuclei (SCN) of the anterior hypothalamus function as such a circadian pacemaker in mammals, generating and regulating numerous circadian rhythms (for reviews, cf. 2,3). This concept was based originally on the results of lesion studies (4) and has been strengthened by the findings that the metabolic (5,6) and electrical activity (7,8) of the SCN vary on a circadian basis. The SCN also manifest a properly timed rhythm of neural activity in culture (9-11). Most recently, fetal SCN transplants have been shown to restore circadian rhythmicity in SCN-lesioned, arrhythmic adult recipients (12). No other discrete area of the mammalian central nervous system has been found that has such circadian pacemaker properties.

Input pathways relay entraining stimuli from the external environment to the circadian pacemaker in the SCN. The most important entrainment pathway is

the monosynaptic retinohypothalamic pathway to the SCN (13-15); this pathway is necessary for entrainment of circadian rhythms to environmental light-dark cycles. An indirect retinal pathway to SCN relays in the ventral lateral geniculate nuclei (16,17) and modulates the entrainment process (18-20).

Output pathways from the SCN are numerous, driving a diverse array of behavioral and hormonal rhythms. The pathway leading from the SCN to the pineal gland, regulating the synthesis of melatonin, is perhaps the most well-delineated output pathway.

DEVELOPMENT OF THE CIRCADIAN TIMING SYSTEM

The development of a functional circadian timing system depends on the maturation of each of its components. In rats, the SCN undergo neurogenesis between days 13 and 16 of gestation (21,22; day 0 = day of sperm positivity). The retinohypothalamic pathway begins innervating the SCN during the immediate postnatal period (23,24), and synapses of retinal fibers with SCN neurons can be identified ultrastructurally on day 6 of age (25). Overt rhythmicity is a postnatal event, with most behavioral and hormonal rhythms not expressed until the second or third weeks of life (for review, cf. 26).

Even though circadian rhythms are not overtly expressed until well into the postnatal period, it is now known that the circadian pacemaker in the SCN is functioning prior to that time. In 1975, Deguchi first provided evidence in rats suggesting that the developing biological clock might be functional and entrained by the mother during fetal life (27). In order to examine the circadian system before the overt expression of rhythmicity, he determined the phase (timing) of the rhythm of pineal N-acetyltransferase (NAT) activity monitored under constant conditions during the postnatal period. Assessment of this postnatal rhythm was used to infer what had happened to the central

oscillator underlying the rhythm at an earlier stage of development. He found that rat pups born and reared from birth under constant conditions express a synchronous population rhythm that is in phase with that of the dam. Furthermore, by fostering pups from birth (under constant conditions) with dams whose circadian time is opposite that of the original dam, the phase of the pups' NAT rhythm shifts toward that of the foster dam. These findings suggested that a circadian clock is oscillating at or before birth and that its phase is influenced by the dam. Since Deguchi's report, several investigators have used a variety of postnatal rhythms to confirm this important finding (for review, cf. 28).

That a circadian clock is actually functioning in utero remained to be shown, however, because of the possibility that some rhythmic aspect of the birth process itself could start and set the timing of the developing clock. Proving that the fetal circadian clock functions before birth requires a method that could measure an intrinsic, functionally relevant property of the clock itself. A method proven useful for monitoring the oscillatory activity of the SCN in adult rats is deoxyglucose (DG) autoradiography (5,6); this method allows for the in vivo determination of the rates of glucose utilization (metabolic activity) of individual brain structures (29).

Reppert and Schwartz applied the DG procedure to study of the SCN in developing rats and demonstrated that an entrainable circadian clock oscillates in the nuclei during late fetal life (30). Furthermore, these investigators showed that ambient lighting acts through the maternal circadian system to coordinate the phase of the fetal clock; the fetal SCN rhythm is always synchronous with the circadian time of the dam and not affected directly by ambient lighting (Fig. 2a). The maternal circadian system continues to coordinate developing circadian phase during the postnatal period

until the developing animal can respond to light directly through its own retinohypothalamic pathway.

SEASONAL REPRODUCTION IN ADULT AND JUVENILE RODENTS

Seasonal variation in environmental conditions occurs throughout temperate regions. Many mammals in these regions undergo physiological changes that increase the likelihood of survival through the harsh winter months. These changes include increasing stores of body fat, growing a thicker but lighter-colored coat, and, in some species, entering hibernation or daily torpor. Many species also restrict mating to portions of the year such that young are born in the spring, when conditions are most favorable for their survival. Daylength (i.e., the length of the light period of the daily light-dark cycle) is probably the most reliable and thus most widely used indicator of season at temperate latitudes.

The photoperiodic regulation of reproduction is the most fully understood of the physiological changes induced by season. In adult Syrian and Djungarian hamsters, long daylengths stimulate or maintain gonadal development; reduction in the daylength below a critical photoperiod inhibits pituitary gonadotropin release (31), inducing testicular regression in males and a cessation of estrous cyclicity in females (32,33). In those species in which it has been examined, the ability to detect changes in photoperiod, and therefore the powerful effect of photoperiod on reproduction, is blocked by pinealectomy (for reviews, cf. 34-37). The involvement of melatonin as the active pineal factor mediating these effects in mammals is indicated by studies using exogenously administered hormone (38-40).

The rate of reproductive maturation in the juveniles of some photoperiodic rodents (e.g., montane voles and Djungarian hamsters) is

affected by photoperiod in much the same way as reproduction is regulated in adults. Short daylengths which suppress gonadotropin release and induce testicular regression in adults also suppress gonadotropins and prevent testicular growth in juvenile males (39,41,42). While critical daylengths have been described for various photoperiod-regulated functions in adults, it is now clear that the response to a photoperiod depends in part on the preceding photoperiodic experience. Daylength is apparently not simply measured, but rather it is measured and compared to what the animal has experienced previously. This presents a problem for juveniles, which are presumably sensing photoperiod directly for the first time. It would clearly be detrimental for juveniles to expend energy in rapidly reaching puberty when the end of the breeding season is imminent. Rather, it would be adaptive for these late-season offspring to invest their energies in preparing for the winter as their parents and other adults will soon be doing. A mechanism for communication of daylength to fetuses (which would allow them to make the seasonally appropriate reproductive response as juveniles) would be of considerable value.

Such a mechanism has been described in montane voles and Djungarian hamsters in which the dam communicates daylength information to the fetus (43,44). Furthermore, the maternal pineal gland and its output signal melatonin are necessary for maternal-fetal communication of daylength (45; Fig. 2b). Maternal-fetal communication of daylength provides the fetus with one daylength measurement, and upon measuring daylength [beginning around postnatal day 15 (46)] the offspring can rapidly make the seasonally appropriate choice between preparing for the summer breeding season or preparing for the winter (e.g., by altering energy metabolism and increasing body fat stores).

PROSPECTUS

In considering environmental factors that must be controlled for studying rodents in microgravity, the lighting conditions in which the animals are housed should take high priority. The evidence cited in this chapter shows that light acting through the circadian timing system profoundly influences several physiological processes. The circadian effects of light are not only important for adult rodents, but are important throughout the early stages of mammalian development.

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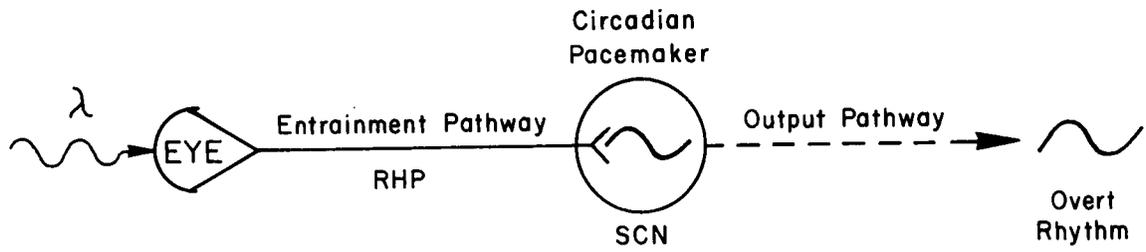


Fig. 1: Major components of the mammalian circadian timing system. RHP, retinohypothalamic projection; SCN, suprachiasmatic nuclei.

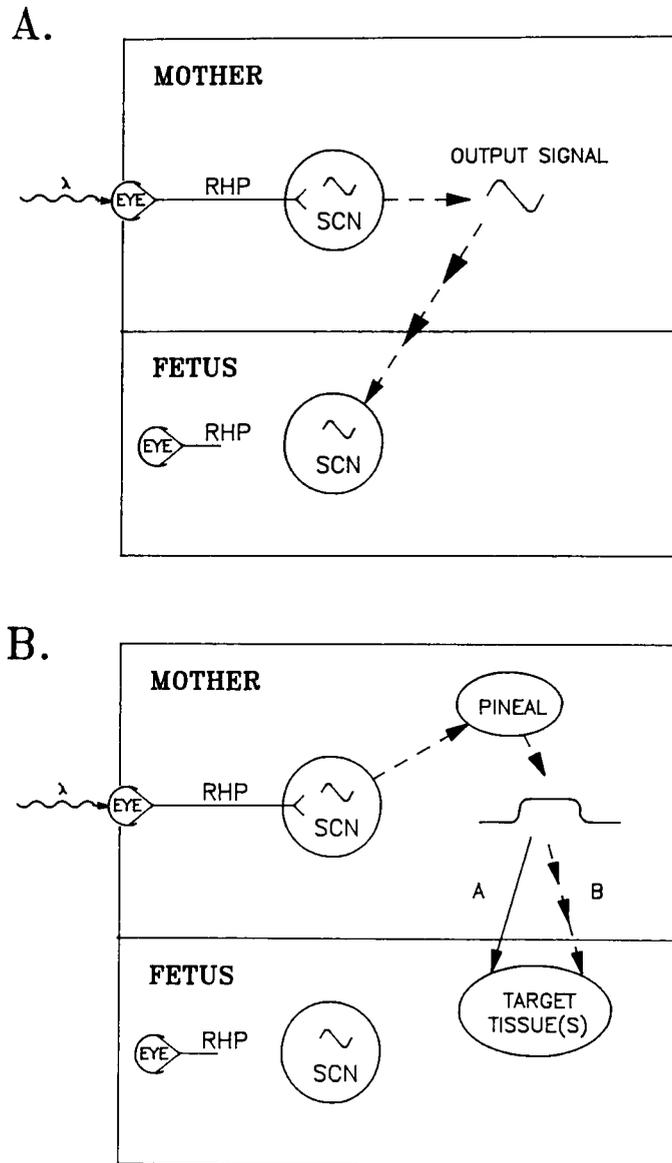


Fig. 2: Conceptual models of maternal-fetal communication of circadian phase (A.) and daylength (B.). A. Light-induced neural signals are conveyed to the dam's SCN by her retinohypothalamic pathway (RHP), entraining her circadian rhythms. A maternal output signal then entrains the fetal clock at a time when the innervation of the fetal SCN by the RHP is incomplete. B. Daylength information is processed by the maternal circadian system leading to a nightly melatonin signal from the pineal gland. The melatonin signal communicates daylength to the fetus by either (a) directly acting on fetal receptive tissues or (b) initiating a cascade of hormonal events in the dam that results in some other signal reaching the fetus.

Light Effects on Non-human Primates

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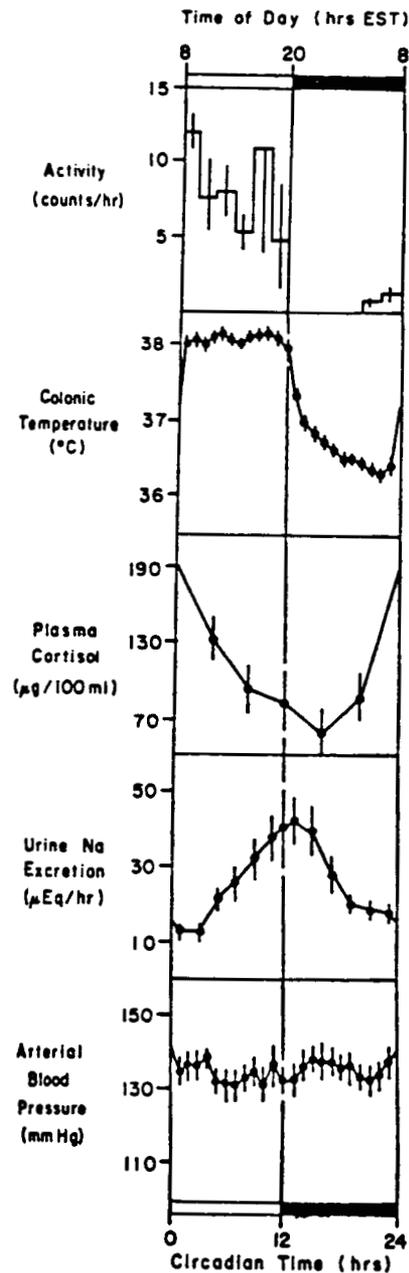
Adaptation to arboreal life has resulted in the stereoscopic and color vision of primates [1]. Given this highly developed visual system, it is not surprising that one of the most important environmental variables for primates is light and dark. The light-dark (LD) cycle has proven to be the most potent synchronizer of circadian rhythms in non-human primates [2]. Entrainment of the circadian rhythms of an organism ensures that the various physiological, biochemical, and behavioral processes of that animal occur at the proper time with respect to the external day and also with respect to each other [3,4,5]. The primate is thus brought into synchronization with its external environment and is, additionally, internally synchronized.

The Circadian Timing System

Figure 1 shows various circadian rhythms in a squirrel monkey [5]. The values are plotted vs. time of day and the animal is in an LD 12:12 (i.e., 12 hours of light followed by 12 hours of darkness). Each curve represents the mean value of several days of data plus the standard error of the mean. Squirrel monkeys are diurnal, and, thus active during the light portion of the LD cycle. Body temperature is high during the light phase and low in the dark. Plasma cortisol peaks around dawn (or lights on), while the opposite pattern is shown by

urinary sodium excretion. No significant rhythm is seen in arterial blood pressure, although many functions that influence blood pressure did show circadian rhythmicity. This animal is not only synchronized to the LD cycle, but all these different functions maintain a stable phase relationship with each other.

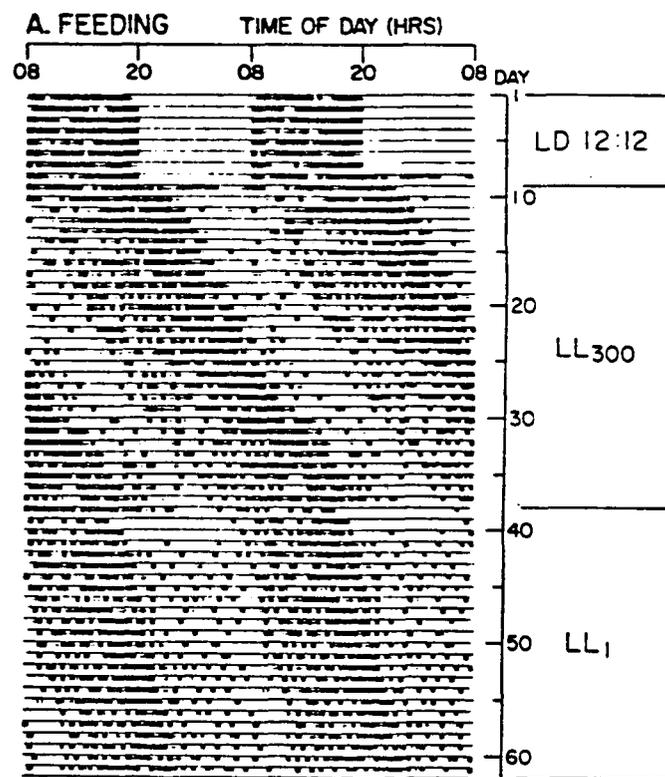
Figure 1: Diurnal rhythms of several physiological functions in squirrel monkeys maintained in an LD 12:12. Each plot shows the average values for a several day period vs. time of day (\pm SEM) [5].



The concept of circadian time is used in order to normalize rhythms between animals with different periods. An animal's day is divided into 24 circadian hours. Activity onset for a diurnal animal (what would have been lights on) is termed circadian time 0. The subjective day runs from circadian time 0 to 12 and the subjective night from 12 to 24 (or 0 of the next circadian day) as shown in Figure 1.

If placed in an environment free of temporal cues, a squirrel monkey will live a day length that is determined by the period of its endogenous clock. Figure 2 shows a double-plotted actograph of drinking data from a squirrel monkey [6]. For the first 8 days of the experiment this squirrel monkey was exposed to a 24 h LD cycle to which it entrained. It was then released into constant light (LL). During this time it lived a day length that was greater than 24 h. The period of this "free-running" monkey was determined by the period of its endogenous clock.

Figure 2: Double plotted actograph for the feeding rhythm in a squirrel monkey. For the first 8 days of the record the monkey was exposed to an LD 12:12 to which it entrained. It was then released into constant conditions. With no temporal cues available, the animal free-ran, living a day length determined by the period of its internal body clock [6].



The Phase Response Curve and Entrainment

Entrainment occurs because the pacemaker itself has a daily rhythm in sensitivity to light. This sensitivity can be elucidated and presented in the form of a phase response curve or PRC [4,7,8,9]. Experimental data can be measured to develop a PRC [3]. For example, a pulse of light is presented to an animal that is free-running in constant darkness. The animal is allowed to continue to free-run after the pulse, and, after 10 days to 2 weeks, the deviation from the expected free-running position is calculated. This change in the position of the rhythm is called a phase shift. The phase shift (in hours) is plotted against the circadian time of the light pulse; by repeating the experiment and giving the pulse of light at different times of the circadian day, a PRC can be constructed (see Fig. 3). Phase advances (movement of the rhythm to an earlier time) are plotted as positive values, while phase delays (movement to a later time) are shown as negative values.

Figure 3 shows a typical mammalian PRC [5]. Light pulses early in the subjective night yield phase delays, while those in the late subjective night give phase advances. Light pulses given during the subjective day are ineffective in eliciting phase shifts and there is a crossover point in the mid-subjective night where no phase shifts occur. It is the sum of the advances and delays caused by light exposure that entrains the animal to the external day-night cycle.

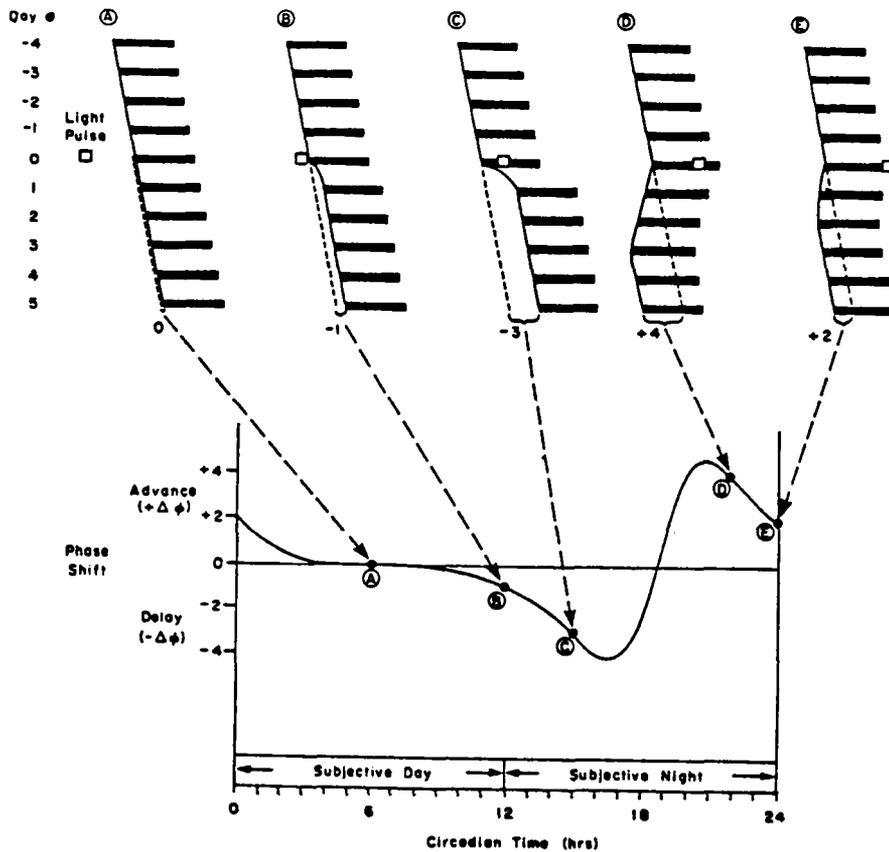
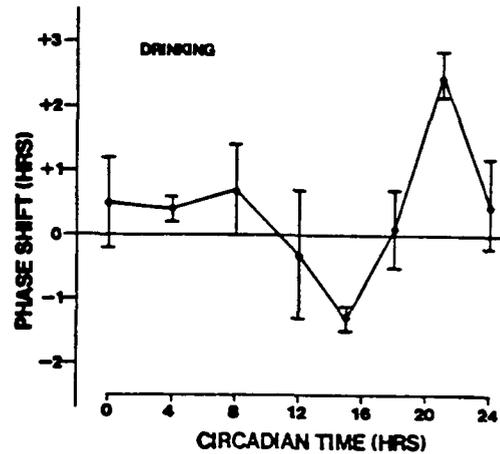


Figure 3: Construction of a phase response curve (PRC). Five experiments showing the results of light exposure at different times of the day are presented. A light pulse given during the subjective day is ineffective in causing a phase shift (A). Light pulses early in the subjective night give phase delays (B & C), while light in the late subjective night causes phase advances (D & E). Plotting the resulting phase shifts against the time of the light pulse gives a PRC [5].

The PRC for one hour light pulses (600 lx) against a background of darkness (0 lx) for a group of 6 squirrel monkeys is shown in Figure 4 [9]. As can be seen, squirrel monkeys show the typical mammalian phase response to light: delays in the early subjective night and advances in the late subjective night. Maximal delays averaged 1.4 h (± 0.2 h), and advances 2.4 (± 0.4 h).

Figure 4: Average phase response curve to 1 h pulses of light for the drinking rhythm in a group of 6 squirrel monkeys (\pm SEM). Maximal average phase advances of 2.4 h were obtained at circadian time 21 and maximal average phase delays of 1.4 h at circadian time 15. This is a normal type 1 phase response curve usually seen in mammals [9].



Light Effects on the Circadian Timing System

Three different aspects of the lighting environment affect the primate circadian timing system. These features include the quality of the light (spectrum and intensity), the quantity (number of hours of light and number of hours of darkness), and the timing (at what point in the animal's day the light and darkness occur).

Spectrum

The spectral composition of the light must be such that it can be perceived by the circadian system. In mammals, thus far, the most potent circadian effects have been seen at a wavelength of 509 nanometers, corresponding to maximum sensitivity of retinal rod cells [10,11]. These circadian effects include both entrainment and phase shifting of activity rhythms.

Intensity

Intensity has effects on both period and waveform. An increase in the intensity of constant light will result in an increase in free-running period. A group of 5 squirrel monkeys free-ran with an average period of 24.3 hours (± 0.07 h) in constant darkness (0 lx). These same animals evidenced a mean period of $25.7 \pm$ h in constant light of 600 lx [9]. It has been hypothesized that this increase in free-running period results from the exposure of the delay portion of the PRC during what would have been the early night. This exposure is then coupled with a loss of exposure of the advance portion of the curve as the animal maintains its usual hours of sleep [12].

A longer free-running period under higher light intensity is also shown by the squirrel monkey in Figure 2 [6]. The period of the feeding rhythm when the animal was exposed to constant light of 300 lx (days 9-38) is longer than that evidenced under exposure to constant light of 1 lx (days 39-62). This can be seen as the larger slope of the 300 lx data compared to the 1 lx data.

The effect of intensity on waveform can be seen in Figure 5 which shows the average waveform for the body temperature, feeding and potassium excretion rhythms of squirrel monkeys in LD 12:12 and in constant light of 1 lx, 60 lx, and 600 lx [13]. Light intensity changes not only the waveform of the resulting rhythm, but also the mean values. Exposure to constant light reduced the 24 h means and circadian ranges of the feeding, temperature and potassium excretion rhythms. Increasing the light intensity also increased the activity-rest ratio (i.e.,

more time is spent at values above the mean than below). The decrease in range of the body temperature rhythm was due to a raising of the minimum temperature achieved by the animal.

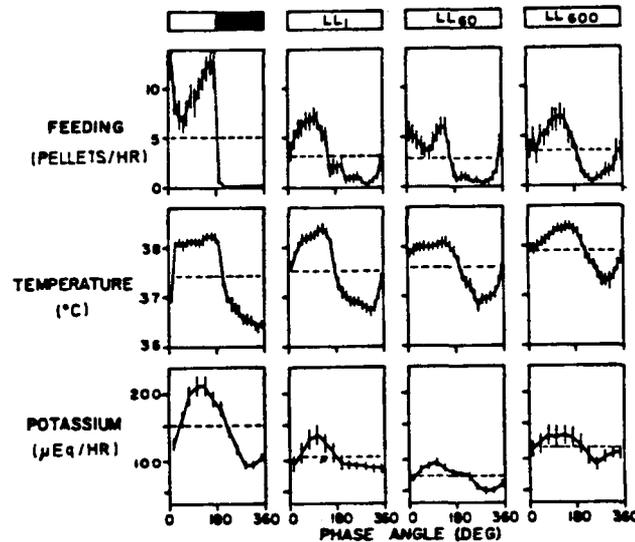


Figure 5: Average waveforms for feeding, body temperature and potassium excretion in squirrel monkeys exposed to an LD 12:12 cycle or to constant light of 1 lx, 60 lx, or 600 lx. Removal of the LD cycle decreased the mean and circadian range of each of these rhythms while increasing the light intensity further decreased the range of the body temperature rhythm through an increase in the minimal body temperature [13].

Further effects of light intensity can be seen in another new world primate, the nocturnal owl monkey. The owl monkey is the only nocturnal simian. Judging from its visual system it is secondarily nocturnal (i.e., it has developed from a diurnal species). It is comparable to the squirrel monkey in size and ecology. Figure 6 shows the average pattern of a week's worth of body temperature and

drinking data from an owl monkey under three different lighting conditions [14]. In all cases, body temperature is presented as a continuous function and drinking as a histogram. In Panel A this animal was entrained to an LD 12:12 cycle (light 100 lx; dark 0.1 lx). By comparing this data with the squirrel monkey rhythms plotted in Fig. 1, you can see that the owl monkey does evidence a nocturnal pattern in these variables (high at night, low during the day). The ratio of 100 lx during the day and 0.1 lx at night proved to give the most robust entrainment in these nocturnal primates [15].

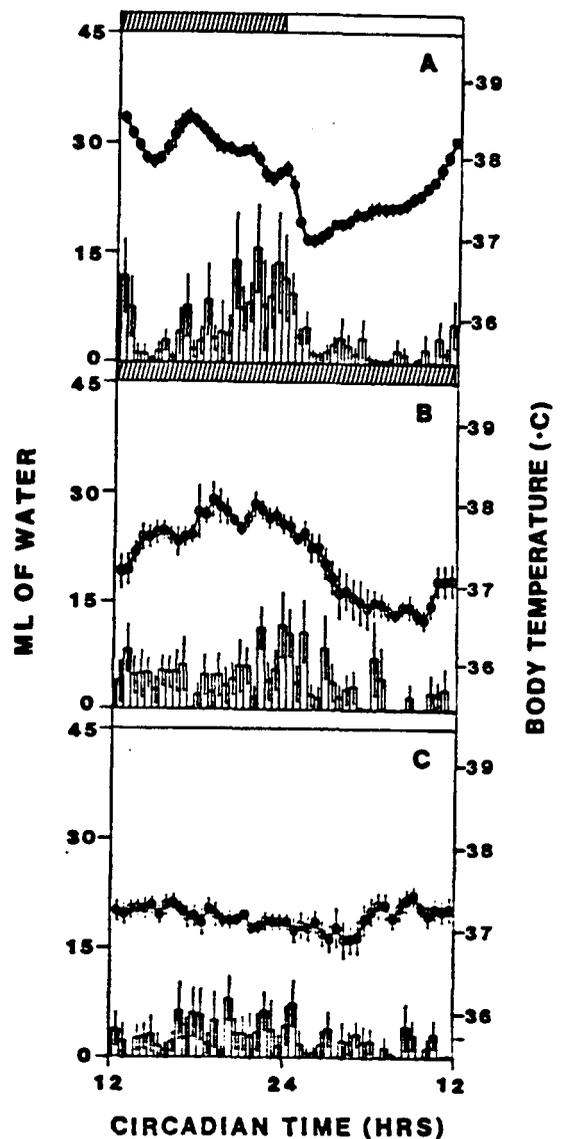
The data presented in Panels B and C emphasizes that light intensity has different effects in different species, and underlines the necessity for absolute control of the experimental lighting environment. Both of these panels show the average waveform from 7 days of data collected from the same owl monkey in constant light. The only difference between the two panels is the light intensity to which the animal was exposed. In Panel B the animal is free-running in constant dim light of 0.1 lx and evidencing a period of 23.5 h. Interestingly, exposure to constant bright light of 100 lx resulted in the disappearance of circadian rhythmicity, as can be seen in Panel C. This can be contrasted with the squirrel monkey who continues to show robust circadian rhythmicity in constant light of 600 lx or more and compared with nocturnal rats which have been shown to lose circadian rhythmicity after 2 to 3 months exposure to LL 200 lx [16].

Figure 6: Average waveforms of body temperature and drinking for an owl monkey in an LD 12:12 ($I_L = 100$ lx; $I_D = 0.1$ lx; Panel A); and constant light of two intensities: 0.1 lx (Panel B) and 100 lx (Panel C). Body temperature is presented as a continuous function and drinking as a histogram.

Panel A: Note the nocturnal pattern of the rhythms, body temperature and drinking are both higher at night than during the day. This can be compared with data from the diurnal squirrel monkey shown in Fig. 1.

Panel B: Under constant dim light (LL 0.1 lx) the monkey evidences free-running rhythms with an average period of 23.5 h.

Panel C: Under constant bright light (LL 100 lx) circadian rhythmicity disappears in this animal, showing its sensitivity to light intensity [14]

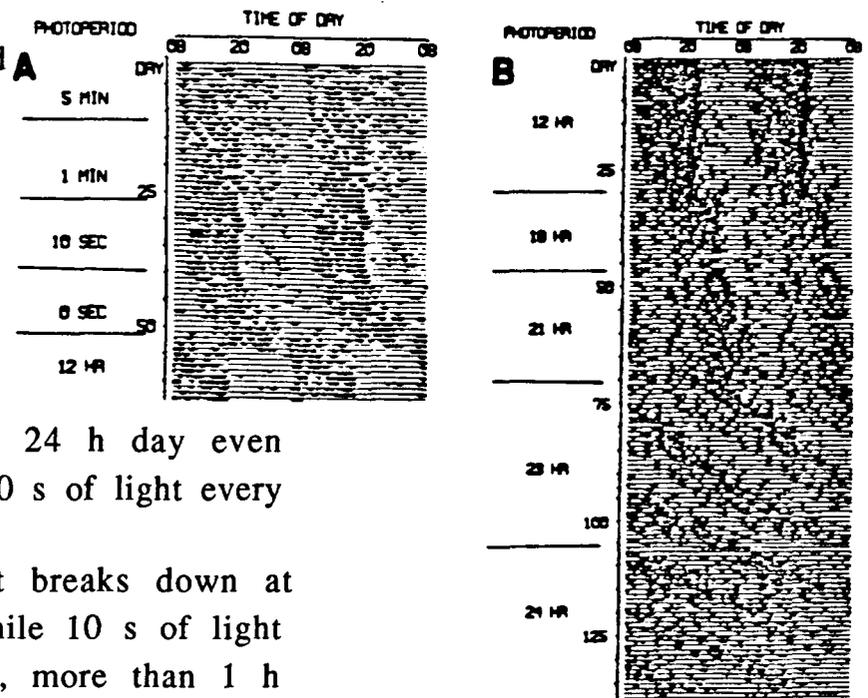


Tonic Effects of Light

The absolute quantity of light and dark presented to a squirrel monkey also has an effect on entrainment. This is illustrated in Figure 7 [17]. Double-plotted actographs of drinking data of squirrel monkeys are presented. The animals in both panels were presented with a 24

hour LD cycle, the differences being that in Panel A the photoperiod (duration of light per 24 h) was gradually shortened, while in Panel B the photoperiod was gradually lengthened. Thus, the monkey in panel A received less and less light per 24 h, while the animal in Panel B received more and more. As can be seen in panel A, squirrel monkeys will entrain to a 24 h LD cycle where the L portion consists of only 10 seconds of light. In contrast, entrainment breaks down when the L portion of the cycle occupies 23 hours of the 24 hour day. While they required as little as one second of light per 24 h for entrainment, these monkeys needed more than 1 h of D per day for entrainment to occur.

Fig. 7: Double-plotted actograph of drinking data from squirrel monkeys in 24 hour LD cycle with either a decreasing (A) or increasing (B) photoperiod.



Panel A: This animal maintains entrainment to the 24 h day even when it receives as little as 10 s of light every 24 h.

Panel B: Entrainment breaks down at an LD 23:1, showing that while 10 s of light are sufficient for entrainment, more than 1 h of dark is necessary per 24 h [17].

Thus far we have been discussing animals that are either free-running in environments with no temporal cues or entrained to the 24 h day. However, squirrel monkeys can be entrained to non-24 h LD cycles. As we have seen in the PRC, the timing of a light pulse can act to cause either a phase advance or phase delay. Thus, in order to entrain a

monkey to a shorter than 24 h day, the light pulse must fall on the phase advance portion of the PRC -- in the late subjective night. In contrast, entrainment to a longer than 24 h LD cycle requires a daily phase delay. This would be caused by light exposure early in the subjective night. In summary, squirrel monkeys that are entrained to a short day will be exposed to light at the beginning of their active period, while monkeys entrained to a long day will experience light at the end of their active period. The length of the entraining cycle determines the relationship between the LD cycle and the monkey's endogenous clock.

Another important aspect of the entraining LD cycle is the quantity of light and dark that is presented. The range of cycle lengths that will elicit entrainment (i.e., the range of entrainment) can be increased, up to a point, by increasing the length of the L portion of the cycle. This can be illustrated by comparing the results of 2 studies examined the range of entrainment in a group of squirrel monkeys. In the first study [9], the animals were exposed to LD cycles that had a photoperiod of one h followed by X number of hours of darkness. Under these conditions, the monkeys entrained to cycle lengths of 23.5 to 26 h (LD 1:22.5 to LD 1:25). Increasing the photoperiod to 3 h doubled the range of entrainment; animals entrained to daylengths of from 22.5 h to 27.5 h [18].

Masking

Light also has direct effects on biological functions separate from its effects on the circadian clock. Such effects are termed masking and may obscure the underlying rhythms. Masking effects in squirrel monkeys include the elicitation of drinking and increase of body

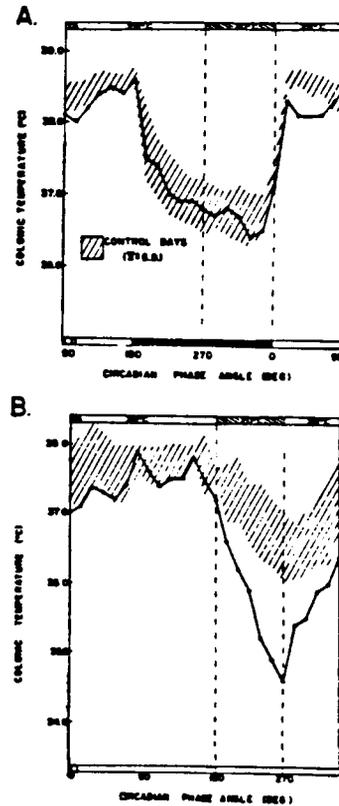
temperature in light and suppression of drinking and decrease of body temperature in dark.

Function of the Circadian Timing System

Throughout this discussion we have been concentrating on the physiological aspects of the CTS, without discussing its importance to the organism. The importance of maintaining entrainment can be seen in the results of an experiment where the thermoregulatory system of a squirrel monkey was challenged [19]. A squirrel monkey entrained to an LD cycle was able to withstand an acute exposure to a cold environment and maintain its body temperature within normal limits (see Fig. 8). However, the cold exposure presented to a free-running squirrel monkey produced a fall in body temperature. The free-running monkey was unable to thermoregulate normally.

Gravity has also been shown to have effects on the circadian timing system. The Biosatellite III monkey showed a reduction in body temperature and a loss in LD entrainment of the temperature rhythm [20]. Loss in entrainment also appeared to have occurred in 2 rhesus monkeys flown on COSMOS 1514 [21] and in rats on Spacelab 3 [22]. Further, squirrel monkeys exposed to hypergravity show an increase in free-running period analogous to the increase in period seen with an increase in light intensity [23].

Figure 8: Effect of 6 h cold exposures on colonic temperature of a squirrel monkey entrained to an LD 12:12 (Panel A) and a monkey free-running in LL (Panel B). The hatched area represents the mean \pm SD of the previous three cycles ($T_a = 28^\circ\text{C}$). As can be seen the entrained animal was able to maintain the integrity of its internal temperature during the cold exposure, while the free-running animal was not [19].



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Recommendations

We have seen that the circadian timing system (CTS) is important for the physiological well-being of an animal. The most important variable affecting the CTS of non-human primates is light. Light also affects the CTS of humans [24,25,26]. In addition, light is important for other systems and interactions are seen between light and gravity. All these observations lead us to make the following recommendations about the experimental lighting environment of microgravity habitats:

-based on our current understanding of the biological influences of light and gravity on the circadian system of mammals, it should be expected that 1G effects of light will likely be different during the

microgravity of spaceflight. Thus, there must be consistency in the lighting environment across missions and across ground experiments.

-the lighting environment must have biological relevance to the organism under study. This generally will mean higher light intensities for diurnal species.

-the experimenter must be able to precisely control light intensity, photoperiod, and duration of the LD cycle.

ACKNOWLEDGMENTS

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**METABOLIC BONE DISEASE; PAST, PRESENT, AND FUTURE:
ROLE OF VITAMIN D AND SUNLIGHT**

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Abstract

Man has worshipped the sun for its life giving properties since the beginning of time. One remarkable biological effect of exposure to sunlight is the production of vitamin D. Although vitamin D, as the name implies, is considered to be a vitamin, it is in fact a hormone. The major physiologic action of vitamin D is to maintain serum calcium concentrations in the normal range in order to maintain neuromuscular function. Vitamin D accomplishes this by increasing the efficiency of the intestine to absorb dietary calcium and to promote the mobilization of calcium from bone. The major source of vitamin D for humans, as well as most vertebrates, is from casual exposure to sunlight. Although some of our food is fortified with vitamin D (in the United States milk is the principle product, whereas in Europe, margarine and cereals are now being fortified with vitamin D), very little of our vitamin D nutrition comes from this source. The focus of this discussion will be on what is known about the importance of sunlight for maintaining vitamin D requirements for mammals and humans, and how the impact of a spacecraft environment will alter this biologic phenomenon. Is it necessary to incorporate into the lighting environment wavelengths between 290 nm and 315 nm in order to promote vitamin D synthesis in the skin, or can vitamin D be supplied by giving astronauts a multivitamin containing 400 international units of vitamin D a day?

Evolution Perspective on Vitamin D

We have evidence that vitamin D was photosynthesized as early as 750 million years ago. This is based on the finding that Emiliani huxleyii, a phytoplankton that has existed on the earth for more than 750 million years, contains high concentrations of provitamin D (1). When this organism was exposed to sunlight, the provitamin D was photolyzed to a precursor of vitamin D known as previtamin D. Additional studies have confirmed these observations and we have recently shown that a variety of zooplankton also photosynthesize previtamin D when they are exposed to sunlight. A detailed analysis of the provitamin D fraction from a high performance liquid chromatography analysis revealed that krill and daphnia contain at least 6 different provitamin Ds. We are now in the process of evaluating the structures of each of these provitamin Ds to determine whether there are some unique compounds that may have important biologic activities in these primitive organisms.

The skin of most fish contains provitamin D₃, and upon exposure to sunlight, the provitamin is converted to previtamin D₃. However, because water is an effective filter for the high energy ultraviolet radiation, it is unlikely that this is the major source of vitamin D for fish. It is likely that fish obtain their vitamin D through the food chain. Most terrestrial plants and animals also have the capacity to produce vitamin D. It was demonstrated at the turn of the century that exposure of lettuce and wheat to high energy ultraviolet radiation would promote antirachitic activity in these substances. We have now found that insects also produce provitamin D, and when exposed to sunlight they convert this precursor to previtamin D₃. An analysis of amphibian and reptile skin has demonstrated that there are large amounts of provitamin D and that there are at least 4 different provitamin Ds.

It should be noted that all vertebrate species require vitamin D to maintain their mineralized skeleton. It is known that if you feed frogs and lizards that have not been exposed to sunlight a steady diet of homegrown insects, they will develop rickets. Birds also require vitamin D, however it was unclear how they were able to make adequate quantities of vitamin D because of the feathers that covered their bodies. In the 1930's, it was suggested

that birds were capable of making vitamin D because they preened themselves. They covered their feathers with an oil from the preen gland that contained provitamin D. During exposure to sunlight the provitamin D on the feathers would be converted to vitamin D which in turn would be either absorbed or ingested. An analysis of chicken feathers, however, has not revealed any provitamin D. When we examined the content of provitamin D in the skin under the feathers as well as on the legs we found that the skin contained about ten times more provitamin D on the legs than on the back. Thus, it appears that the chicken is able to regulate provitamin D synthesis in areas that are exposed to sunlight in order to maximize the cutaneous production of vitamin D.

An examination of aquatic mammalian skin revealed 3 to 4 different provitamin Ds. It is well known that polar bears have a high content of vitamin D in their livers. It has always been assumed that this is principally coming from the diet. Although this is probably true, it should be noted that the polar bear hair follicle is hollow and acts like a fiber optic system. It is possible that during the summer this fiber optic system enhances the cutaneous photosynthesis of vitamin D.

Therefore, based on our observations, it would appear that most plants and animals that are exposed to sunlight have the capacity to produce vitamin D. We were curious as to why phytoplankton and zooplankton would make vitamin D since, obviously, there is not a need for this compound for regulating calcium metabolism in unicellular organisms that live in the high calcium environment in the oceans. There may be another reason why provitamin D became so important in early evolution. As unicellular organisms developed the use of sunlight to promote the photosynthesis of carbohydrates, they were confronted with a major problem. Exposure to visible radiation was required to promote the photosynthesis of sugars. However, sunlight also contained high energy ultraviolet radiation that could damage important macromolecules such as DNA, RNA, and proteins. These organisms would have had to develop a natural suncreening mechanism that would permit visible radiation to enter the organism while absorbing the harmful ultraviolet radiation. The provitamin D may be one of the compounds used as a natural sunscreen. The provitamin D absorbs radiation from 240 nm up to 320 nm. When phytoplankton were exposed to sunlight, the high energy photons, up to 320 nm, converted provitamin D to previtamin D (Fig.1). Once formed, previtamin D can absorb an additional photon of high energy radiation and isomerize to lumisterol or tachysterol. These isomers, in turn, could absorb high energy ultraviolet radiation and isomerize to previtamin D. Thus, provitamin D and its photoproducts can act as a high energy photon sink. Because these products do not absorb visible radiation, they permit this radiation to penetrate into the organism for photosynthesis of carbohydrates. However, provitamin D and its photoproducts are effective in absorbing the high energy ultraviolet radiation that potentially could damage and destroy many of the sensitive and important macromolecules of the unicellular organisms. In addition, when provitamin D is photolyzed, the amount of photoproducts that are generated are an indication of the total amount of ultraviolet radiation that the organism was exposed to. It may be that these photoproducts provided a message to them regarding the amount of solar radiation that they have been exposed to. Thus, early in evolution, provitamin D may have evolved as a sunscreen and may have acted as a actinometer as well (1).

Regarding human history, the vitamin D story begins in the 17th century. As the rural populations began to congregate in centers in Northern Europe and as the industrial revolution began to take grip in these cities, tall dwellings were built in close proximity to each other and the atmosphere was fouled by industrial pollution. As a result, essentially no sunlight penetrated into the

alleyways where the children were playing. As early as 1650, it was appreciated by Whistler, DeBoot, and Glisson that these children developed a very severe bone disease which they identified as a distinct clinical entity. This disease, commonly known as English disease or rickets, was identifiable in children based on deformities of the skeleton including bowed legs and hypertrophy of the costochondral junctions along the rib cage (rachitic rosary), as well as muscle weakness. As early as 1822, Sniadecki realized that children living in Warsaw, Poland, had a high incidence of rickets, whereas children living in the rural areas outside of Warsaw did not. He concluded that it was likely that the lack of exposure to sunlight to the children living in the city was the cause for the disease (2). Seventy years later, Palm reported that it was likely that sunlight was important for the prevention and cure of rickets based on his epidemiologic survey (3). He found that rickets was rare in children in China, Japan, and India where their nutrition was poor; whereas, the disease was rampant in the industrialized cities in Great Britain. He hypothesized that there was a photochemical reaction that occurred in the skin that was responsible for preventing and curing rickets. He suggested the removal of rachitic children as soon as possible from the inner cities so that they could be exposed to the healthful effects of sunlight. He advocated the education of the public for the appreciation of sunshine as a means of health.

However, it was inconceivable at this time that exposure to sunlight could have any significant importance for preventing bone disease. As a result, these intuitive observations were considered to be without merit. By the turn-of-the-century, scientists from all walks of life became involved in trying to understand what caused rickets. Many scientists believed that it was an inherited disorder and could not be cured. Others, however, considered that it was lack of activity or possibly an infection that caused the disease. It was well known that cod-liver oil could cure this disease and thus the antirachitic factor was considered to be a nutrient or a vitamin. Findlay was convinced that it was lack of activity that caused rickets. He put rodents in a glass box that was just big enough to hold the animals so that they were unable to move. He demonstrated that these animals developed rickets and concluded that sunlight did not have a curative effect on rickets but that it was lack of activity that was the major etiologic factor. He had not appreciated at that time that the glass absorbed the high energy radiation that was responsible for making vitamin D in the skin.

In 1919, Huldshinsky exposed four rachitic children to radiation from a mercury vapor arc lamp and showed radiologically that rickets could be cured within 4 months (4). Two years later, Hess and Unger (5) exposed 8 rachitic children to sunlight on the roof of a New York City Hospital and demonstrated that their rickets was cured within several months (5). Based on these and other observations it was concluded that irradiation by sunlight or high energy ultraviolet radiation could impart antirachitic activity.

Steenbock and Black (6), and Hess and Weinstock (7), independently showed that irradiation of a variety of substances including vegetable matter, cotton seed oil, serum, and laboratory rat food, imparted antirachitic activity to these substances. These observations prompted the fortification of milk with the provitamin D, and then the irradiating of it with high energy ultraviolet radiation. Once the structure of vitamin D was known and the vitamin was chemically synthesized, vitamin D was added directly to milk. In the United States a quart of milk contains the recommended daily allowance of vitamin D which is 400 international units (10 micrograms). Although it is appreciated that vitamin D is essential for the growth and maintenance of a healthy skeleton in children, it is not often realized that vitamin D is absolutely essential for the development and maintenance of our skeleton throughout our

entire lives (8).

Synthesis of Vitamin D in Human Skin

During exposure to sunlight, the high energy radiation with wavelengths between 290 nm and 315 nm penetrates into the epidermis and converts epidermal stores of provitamin D₃ (7-dehydrocholesterol) to previtamin D₃ (Fig. 1)(8-11). In adult skin 50% of the provitamin D₃ is found in the epidermis and the other half is found in the dermis. However, when adult skin is exposed to sunlight the ultraviolet B portion (290 nm - 320 nm) of the solar spectrum that passes through the atmospheric ozone layer is absorbed in the epidermis, and insignificant amounts of these photons penetrate into the dermis. As a result, greater than 80% of the previtamin D that is formed in human skin occurs in the epidermis. There is approximately 2 to 8 micrograms/cm² of provitamin D in the epidermis. This concentration is related to age.

Once previtamin D₃ is photosynthesized in the epidermis, this biologically inert photoproduct begins to isomerize by a temperature-dependent process to vitamin D₃ (Fig. 1). This process takes approximately three days to reach completion. Once formed, vitamin D₃ is translocated from the epidermis into the dermal capillary bed by the action of the vitamin D binding protein which is found in the circulation.

There are several factors that can diminish the cutaneous production of vitamin D₃. There is an inverse relation between the concentrations of provitamin D₃ in the epidermis with age (11,18). Compared with elderly adults, young adults can make 2 to 3 times more previtamin D₃ in their skin. Sunscreens which are effective in preventing the damaging effects of sunlight also prevent the beneficial effect of sunlight, the photosynthesis of previtamin D₃ in human skin (19). The topical application of a sunscreen with a sun protection factor of 8 can completely block the photosynthesis of previtamin D₃. The natural sunscreen that is produced by the skin, melanin, also acts as an effective neutral filter and absorbs wavelengths of sunlight that are responsible for producing previtamin D₃ (20). Loomis had suggested that skin pigmentation evolved for the purpose of regulating cutaneous vitamin D synthesis in the skin (12). He proposed that people living at or near the equator would have died of vitamin D intoxication as a result of daily exposure to intense solar radiation were it not for the evolution of more melanin pigmentation in the skin. Although melanin will compete with provitamin D for high energy solar photons, and therefore limit the skin's ability to produce vitamin D₃, this is not the major regulatory mechanism by which vitamin D synthesis is controlled. It is now appreciated that there is a more fundamental process that regulates the formation of previtamin D in human skin. Previtamin D₃ is sensitive to both thermal energy and ultraviolet radiation. Once previtamin D₃ is formed in the skin, it can either thermally isomerize to vitamin D₃ or absorb a photon of ultraviolet radiation and isomerize to the biologically inactive isomers, lumisterol and tachysterol (Fig. 1 and 2) (13). Thus, during the initial exposure to sunlight, provitamin D₃ is converted to previtamin D₃. During prolonged exposure to sunlight, previtamin D₃ is photolyzed principally to lumisterol, and to a small extent, tachysterol (Fig. 1 and 2). Thus, prolonged exposure to sunlight will not increase previtamin D₃ concentrations in human epidermis above approximately 10% of the original provitamin D₃ concentration. It is the photochemical degradation of previtamin D₃ that is the major factor that limits the production of this vitamin D precursor. It is the component parts of the solar spectrum that limits the overall conversion of provitamin D₃ to previtamin D₃ (10). If human skin is exposed to monochromatic radiation of wavelengths 295 ± 5 nanometers, the

photochemistry can be altered to the extent that 60% to 70% of provitamin D₃ is converted to previtamin D₃ (Fig. 3) (10). The mechanism for this increased production is related to the ultraviolet absorption spectra of the isomers and quantum efficiencies for the photochemistry of provitamin D₃ and its various photoproducts (Fig. 4) (10).

Once formed in the skin, it also is susceptible to sun induced photodegradation. Recently, it was found that vitamin D₃ is more sensitive to exposure to sunlight than is previtamin D₃ (14). For example, on a sunny day in June approximately 80% of the cutaneous stores of vitamin D₃ are destroyed within 1 hour, and after 3 hours no detectable vitamin D₃ is present. The principle products that are formed from the photodegradation of vitamin D₃ are 5,6-transvitamin D₃, suprasterol 1, and suprasterol 2. The physiologic roles of these isomers of previtamin D₃ and vitamin D₃ are unknown at the present time.

It has always been assumed that human skin contains only one provitamin D, provitamin D₃. However, based on our evolutionary biology studies, we examined the possibility that human skin may contain more than one provitamin D. A careful analysis of a lipid extract from human and rat skin by high performance liquid chromatography has revealed the presence of an additional provitamin D that has been structurally identified as 24-dehydroprovitamin D₃ (15). Although the physiologic significance of 24-dehydroprovitamin D₃ remains unknown, it is interesting that its photoproduct, 24-dehydrovitamin D₃, is a potent inhibitor of the rat liver microsomal, vitamin D 25-hydroxylase (16).

Therefore, when considering the lighting environment for astronauts and animals for long duration space flight, it might be important to be aware of the differential effects of different wavelengths of radiation on the photochemistry of provitamin D₃, previtamin D₃, and vitamin D₃. In addition, it should be appreciated that exposure to sunlight produces two vitamin Ds in human skin. The addition of vitamin D₃ to the diet would not be able to substitute for cutaneous production of 24-dehydrovitamin D₃. To date, however, there is no evidence that 24-dehydrovitamin D₃ is essential for human health. Furthermore, we now have over 80 years of experience with vitamin D fortification of food, and it appears that vitamin D fortification is more than adequate to maintain all of the biological actions of vitamin D₃ in humans.

Influence of Season and Latitude on the Cutaneous Production of Previtamin D₃.

Because casual exposure to sunlight is important for providing our vitamin D requirement, it was of interest to determine the role of season and latitude on the cutaneous production of previtamin D₃. We developed a model whereby we exposed tritium labeled provitamin D₃ in an organic solvent and human skin to sunlight on the roof of our institution in the middle of the month on cloudless days throughout an entire year. In June, we found that after 10 minutes of exposure approximately 2% to 3% of provitamin D₃ was converted to previtamin D₃. After 2 hours, approximately 10% of the provitamin D₃ was converted to previtamin D₃ and further exposure did not increase previtamin D₃ production but rather generated lumisterol and tachysterol. Cutaneous synthesis of previtamin D₃ was found to occur between the months of March through October, however, exposure of up to 5 hours of sunlight in Boston during the months of November through February did not produce any previtamin D₃ in human skin or in our *in vitro* model. Similar studies were conducted in Edmonton, Canada, which is 10° north of Boston (52° north). It was found that the vitamin D winter period extended between the months of October through March. These observations are potentially important, especially for the elderly who depend on sunlight for most of their vitamin D requirement. During the period of

time when cutaneous synthesis of previtamin D_3 cannot occur, it is recommended that a supplement of vitamin D containing 400 international units be ingested.

Vitamin D Metabolism

Once vitamin D is made in the skin or absorbed from the diet, it enters into the circulation and is transported to the liver where it undergoes a hydroxylation on carbon 25 to produce the major circulating form of vitamin D, 25-hydroxyvitamin D_3 (Fig. 4) (21,22). Although aging decrease the capacity of human skin to produce vitamin D_3 , it does not alter the absorption of vitamin D from the diet. Once 25-hydroxyvitamin D_3 enters the circulation, it travels to the kidney where it is hydroxylated by a mitochondrial enzyme complex (cytochrome P-450) to form the biologically active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Fig. 4).

1,25-dihydroxyvitamin D_3 travels to the intestine and interacts with a nuclear receptor to unlock genetic information which is responsible for enhancing the efficiency of intestinal calcium absorption. This hormone also is transported to the bone and in concert with parathyroid hormone, enhances the mobilization of calcium stores from this tissue (21-23). Thus, the major purpose of $1,25(OH)_2D_3$ is to maintain circulating concentrations of calcium within the normal range in order to maintain neural and muscular function. Although vitamin D is recognized to be important for bone mineralization, there is mounting evidence that $1,25(OH)_2D_3$ does not actively participate in bone mineralization. Instead, it acts indirectly by increasing bone fluid calcium and phosphorus levels to suprasaturation concentrations that are then deposited in the bone matrix by a passive event.

As expected, the intestine, bone, and kidney were found to possess nuclear receptors for $1,25(OH)_2D_3$. In 1979 Stumpf et. al., demonstrated the nuclear localization of $1,25(OH)_2D_3$ in such diverse tissues as stomach, pituitary glands, parathyroid glands, skin, and gonads (26). It is now known that most of the tissues in the human body possess high affinity, low capacity receptors for $1,25(OH)_2D_3$. Although significance of these observations remains to be determined, it has been demonstrated that a variety of tumor cells that possess receptors for $1,25(OH)_2D_3$ are very sensitive to this hormone. For example, HL-60 human promyelocytic leukemic cells, when incubated with $1,25(OH)_2D_3$, stopped proliferating and matured into functional macrophages (27). Peripheral circulating monocytes and bone marrow monocytes also possess receptors for $1,25(OH)_2D_3$. When these cells are exposed to this hormone they begin to mature into multinucleated forms that have the capacity to mobilize calcium-45 from bone. These observations have prompted speculation that $1,25(OH)_2D_3$ may have a very important physiologic action on the mobilization of stem cells to form osteoclasts (24). Indeed, recent evidence suggests that mature osteoclasts do not possess receptors for $1,25(OH)_2D_3$ (25). It appears that this hormone is capable of increasing the mobilization of calcium from bone indirectly by mobilizing stem cells to become osteoclasts (25).

There are a variety of practical applications to these new biological actions of $1,25(OH)_2D_3$. For example, human epidermal cells possess receptors for this hormone and when exposed to $1,25(OH)_2D_3$ in culture, these cells stop proliferating and mature into normal epithelium. Based on these observations there has been recent evidence that $1,25(OH)_2D_3$ may be of pharmacological value for the treatment of hyperproliferative disorders of the skin such as psoriasis (28,29).

Although circulating resting lymphocytes do not possess receptors for $1,25(OH)_2D_3$, when these cells are activated they generate a receptor for $1,25(OH)_2D_3$. This hormone will decrease interleukin 2 and immunoglobulin

synthesis in activated T and B lymphocytes, respectively.

Although it appears that $1,25(\text{OH})_2\text{D}_3$ may have important biologic activities independent of its role on regulating calcium homeostasis, it should be appreciated that patients with a rare hereditary disorder known as vitamin D dependent rickets type II (these patients either lack or have a defective receptor for $1,25(\text{OH})_2\text{D}_3$) have normal immune and endocrine functions. Therefore, the physiologic role of $1,25(\text{OH})_2\text{D}_3$ on non-classical target tissues may be very subtle and its importance remains to be determined.

Metabolic Bone Disease: Past, Present and Future

Aging is associated with a decrease in bone mass. This process, whereby there is a loss of bone mineral and bone matrix, is called osteoporosis (8,21). This disease afflicts approximately 20 million elderly Americans and is responsible for the 1 million fractures that are seen in the population each year in the United States. It is not often recognized that there is another disease of bone that is related not to age, but to vitamin D deficiency. Vitamin D deficiency in children causes rickets, whereas vitamin D deficiency in adults causes a bone disease known as osteomalacia. Osteomalacia is caused by a mineralization defect of normal bone matrix. When a person becomes vitamin D deficient, there is an increase in the secretion of parathyroid hormone which in turn increases the mobilization of calcium from bone. Therefore, initially vitamin D deficiency will enhance the destruction of bone and increase the degree of osteoporosis. In addition, the defect in bone mineralization makes the skeletal structure weaker and may be responsible for the 40% incidence of hip fracture in males and females that are vitamin D deficient (8). If astronauts and experimental animals are not obtaining adequate vitamin D nutrition during prolonged space flight, they have the potential of enhancing zero gravity bone loss and developing a mineralization defect of newly formed osteoid.

Conclusion

In 1650 humans were concerned about rickets; in 1987 the bone disease of most concern is osteoporosis in the aged. Probably in 2020 as we begin long duration space exploration, zero gravity bone loss will be of great importance. The role of vitamin D for maintaining adequate bone mineralization is well documented and it will be important for astronauts and laboratory vertebrate animals to receive adequate vitamin D nutrition. This can be provided either by dietary supplementation or by designing ultraviolet radiation with wavelengths of 290 nm to 315 nm in the lighting environment. This radiation will promote the cutaneous production of vitamin D_2 . It will also promote the cutaneous production of 24-dehydrovitamin D_3 . Whether vitamin D supplementation in the diet will completely replace the biologic functions of the vitamin Ds that are produced in the skin is open for speculation. From my perspective, it is not unreasonable to incorporate in the lighting environment for prolonged space flights, a small amount of UVB (290 nm-315 nm) ultraviolet radiation. It should be appreciated that if the lighting environment only contains UVA radiation between 340 nm-360 nm, that this radiation could potentially degrade circulating vitamin D and its metabolites. There is no question that for short duration space flights, dietary vitamin D is adequate. This may not be true, however, for a long duration space flight.

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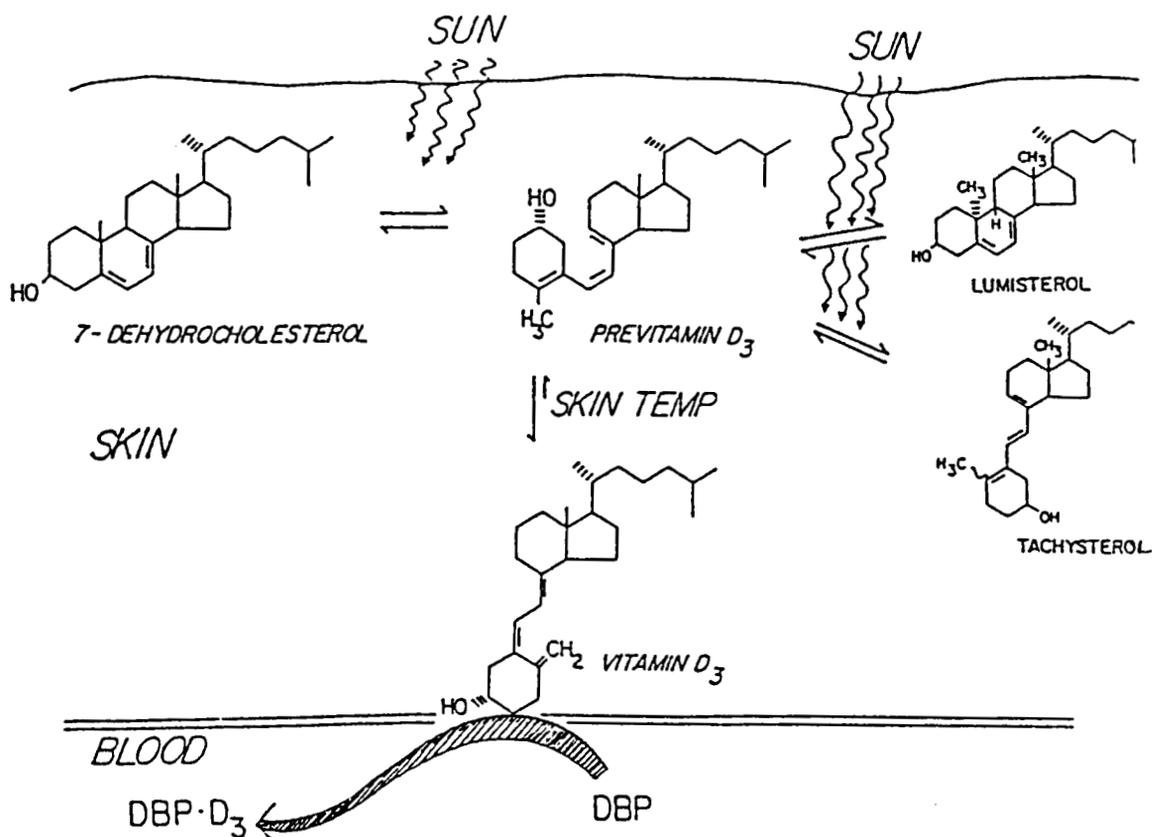


Figure 1. Schematic representation of the formation of previtamin D₃ in the skin during exposure to the sun and the thermal isomerization of pre-D₃ to vitamin D₃. Vitamin D₃ is specifically translocated by the vitamin D binding protein (DBP) into the circulation. During continual exposure to the sun, previtamin D₃ also photoisomerizes to lumisterol₃ and tachysterol₃, which are photoproducts that are biologically inert (i.e., they do not stimulate intestinal calcium absorption). Because the DBP has no affinity for lumisterol₃ but has minimal affinity for tachysterol₃, the translocation of these photoisomers into the circulation is negligible, and these photoproducts are probably sloughed off during the natural turnover of the skin. Because these photoisomers are in a state of quasi-photoequilibrium as soon as previtamin D₃ stores are depleted (due to thermal isomerization to vitamin D₃), exposure of lumisterol and tachysterol to UV radiation will provoke these isomers to photoisomerize to pre-D₃. (From Holick et al. 13 with permission. Copyright 1981 by the American Association for the Advancement of Science.)

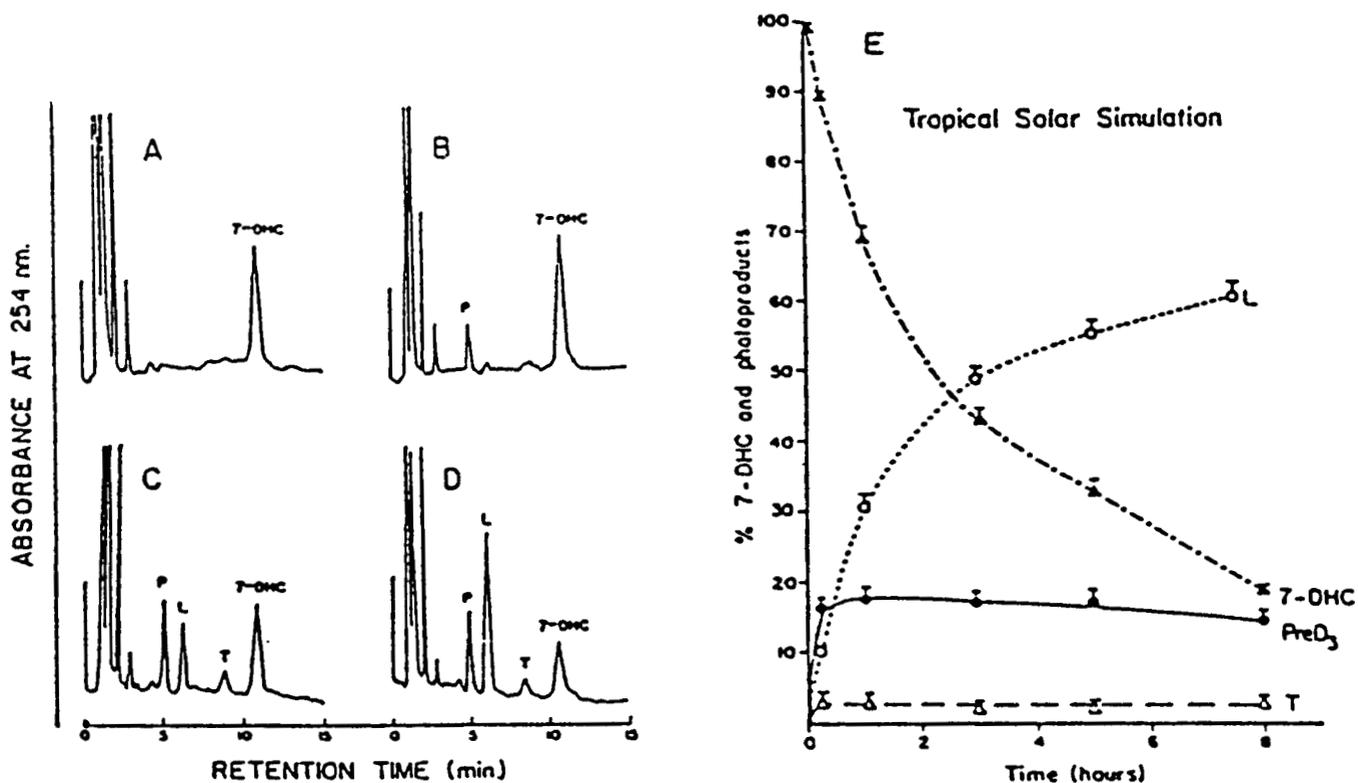


Figure 2. HPLC profiles of a lipid extract from the basal cells of surgically obtained hypopigmented skin that was previously shielded from (A) or exposed to (B-D) equatorial simulated solar ultraviolet radiation for 10 min (B), 1 hr (C), or 3 hr (D). E, an analysis of the photolysis of 7-dehydrocholesterol (7-DHC) in the basal cells and the appearance of the photoproducts previtamin D₃ (pre-D₃), lumisterol (L), and tachysterol (T) with increasing time of exposure to equatorial simulated sunlight. From Holick et al. 13 with permission. Copyright 1981 by the American Association for the Advancement of Science.

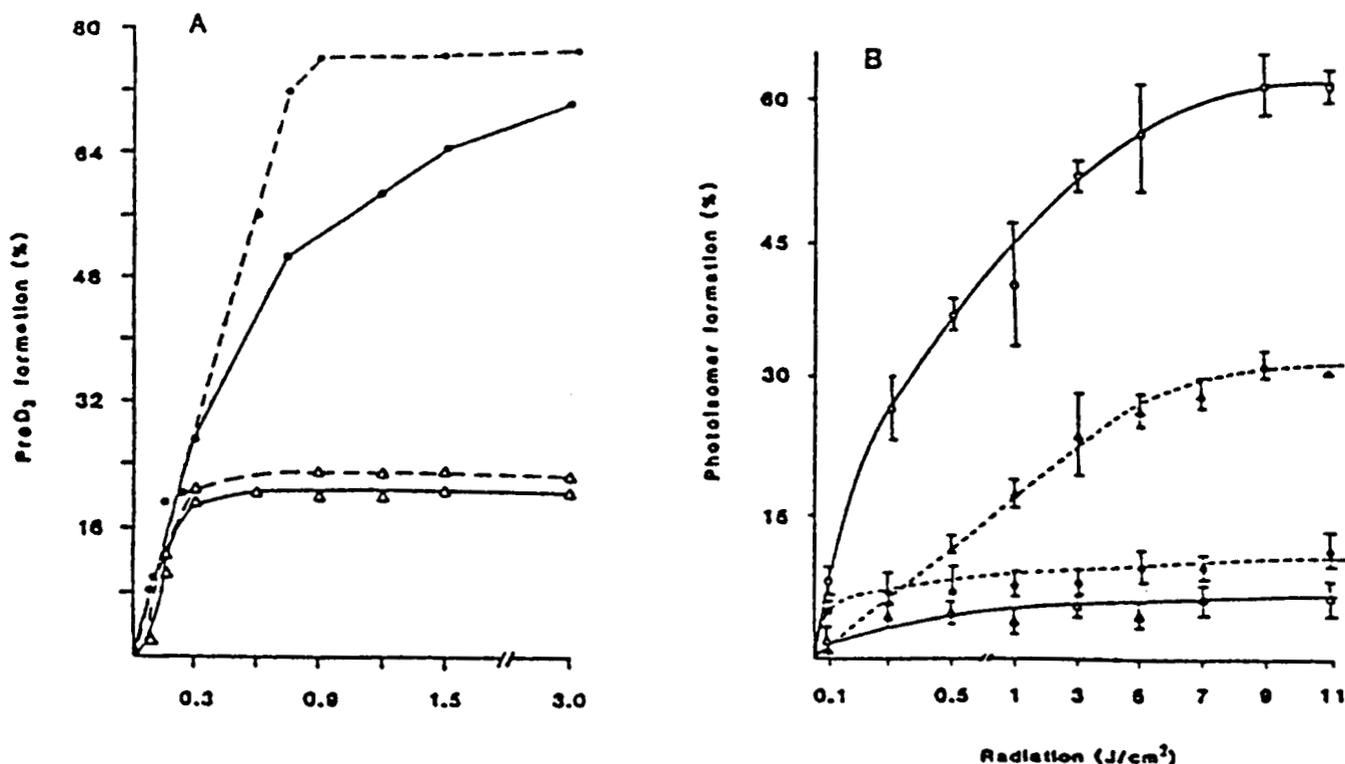


Figure 3. (A) Percent formation of preD₃ from 7-dehydrocholesterol (7-DHC) in human epidermis (—) or from crystalline 7-DHC (10 ug/ml) dissolved in tetrahydrofuran (----) after exposure to (●) a range of doses of narrow-band radiation at 295 ± 5 nm (1) or to (△) simulated solar radiation (2). (B) Percent formation of lumisterol (○ and ●) and tachysterol (△ and ▲) in human epidermis after exposure to a range of doses of narrow-band radiation at 295 ± 5 nm (----) or to simulated solar radiation (—) (2). The amount of UV is measured by a 295-nm radiometer for the narrow-band source, and the amount of 290 to 302 nm radiation is measured by a radiometer for the simulated solar radiation source.

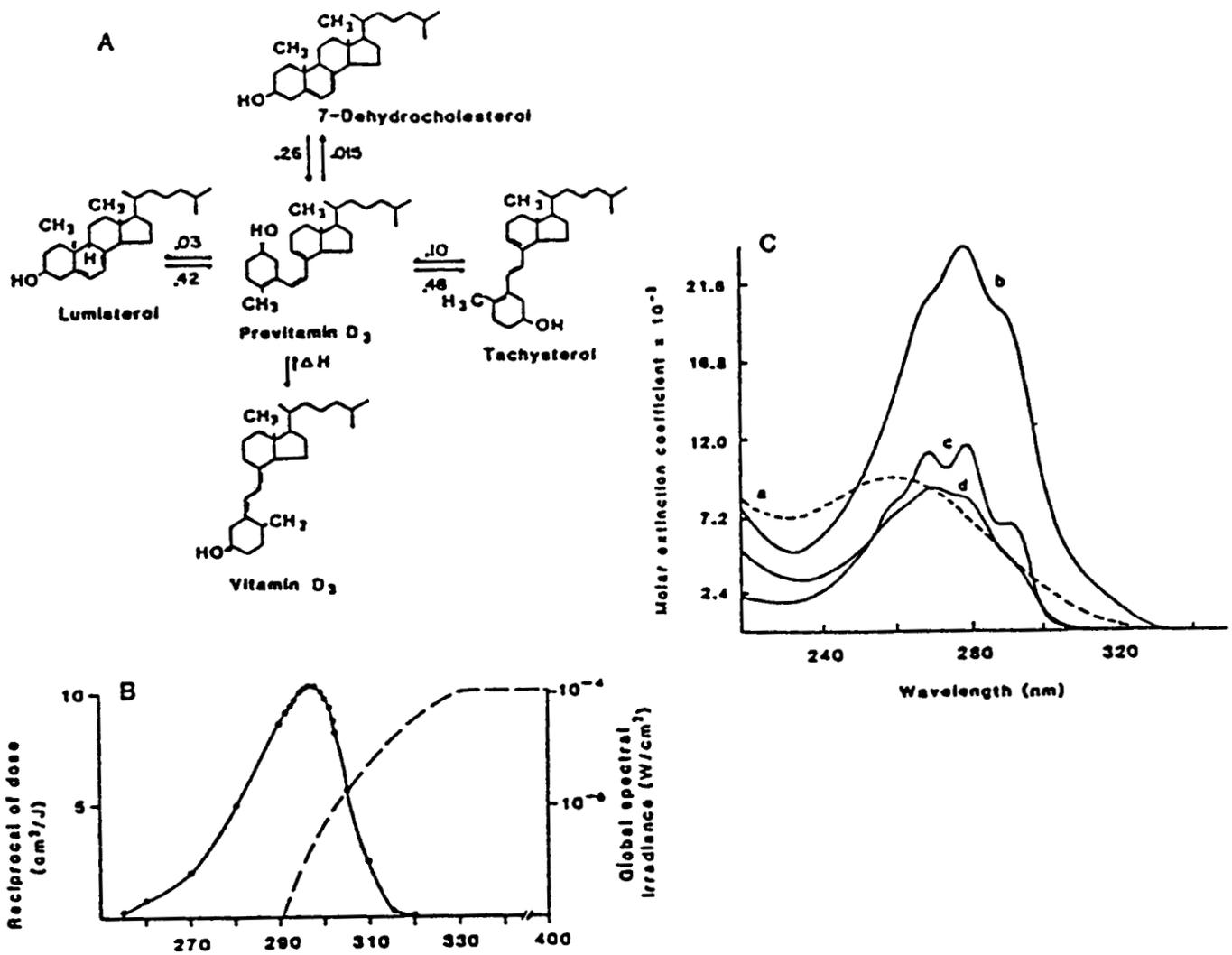


Figure 4. (A) The photochemical reaction in human epidermis of 7-DHC to preD₃. The preD₃ either thermally converts to vitamin D₃ or photoisomerizes to lumisterol₃ and tachysterol₃. (B) The action spectrum of preD₃ formation from 7-DHC in human epidermis (O) and the spectral irradiance curve for sunlight (---). The action spectrum was obtained by plotting the reciprocal of the dose as a function of wavelength. At any wavelength, no more than 5 percent of product was made. The overlay of the curve of the action spectrum with that of the solar spectrum demonstrates the small portion of the solar UV spectrum that is involved with the production of preD₃ from 7-DHC. (C) Ultraviolet absorption spectrums for (a) preD₃, (b) tachysterol₃, (c) 7-DHC, and (d) lumisterol, isolated from human epidermis.

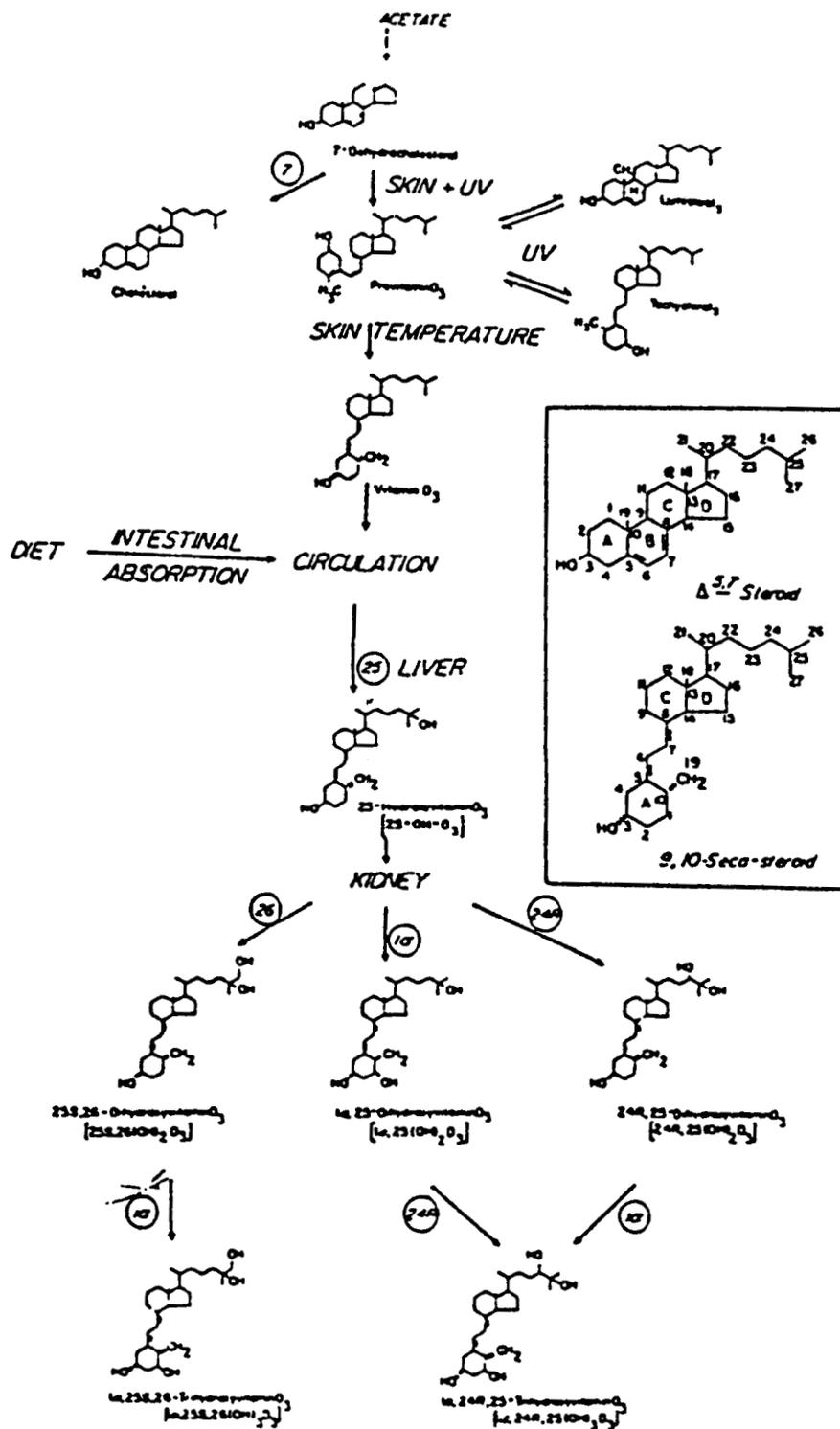


Figure 5. The photochemical, thermal, and metabolic pathways for vitamin D₃. Circled letters and numbers denote specific enzymes: ⑦ = 7-dehydrocholesterol reductase; ②⑤ = vitamin-D-25-hydroxylase; ① = 25-OH-D-1 α -hydroxylase; ②④R = 25-OH-D-24R-hydroxylase; ②⑥ = 25-OH-D-26-hydroxylase. From MF Holick and JT Potts, Jr. Vitamin D, in Isselbacher KJ, Adams RD, Braunwald E, Petersdorf RG, Wilson JD (eds): Harrison's Principles of Internal Medicine (ed. 10). New York: McGraw-Hill Book company, 1983:1944-1949. Reproduced with permission.

LUMINANCE AND CYCLIC STIMULATION IN MAMMALIAN ONTOGENESIS

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The design and implementation of animal habitats for the microgravity conditions of space is a fascinating challenge that brings together the concerns and requirements of animals, scientists, and engineers. The present chapter is a brief, selective overview of mammalian ontogenesis, with emphasis on the roles of light and other cyclic features of the environment. The purpose is to provide a fuller appreciation for the regulation by light of behavior and physiology during animal development, and thus to identify known or likely biological issues that will be part of successful animal habitats for microgravity research.

Light's behavioral and physiological effects can be both direct and indirect. Direct effects of light are typically those in which photic stimulation (or its periodicity) alters organismic function via a photosensitive pathway. In animals direct effects of light are often, though not exclusively, mediated via retinal pathways. Indirect effects of light are those that derive from photically-driven processes, although the immediate causal stimulus is not necessarily light. For instance, as will be seen later in my discussion, the development of mammalian offspring is particularly susceptible to indirect effects of light because the mother can be (directly) affected by

light and these direct effects can then be expressed indirectly on the offspring through her behavior and physiology.

Throughout the present chapter I will discuss rodent development, specifically that of the domesticated Norway rat (Rattus norvegicus). This focus is directly relevant to NASA's interests in habitat design, for Norway rats are among the premier specimens both for basic and for biomedical research in space. Furthermore, the conceptual approach that I apply for ontogenetic analysis is, in fact, rather broadly applicable for consideration of other mammalian species (see Alberts & Cramer, 1987).

The seminal contributions of Professor Curt Richter should be noted at the outset. His influence pervades many, if not most facets of the research reviewed in this chapter.

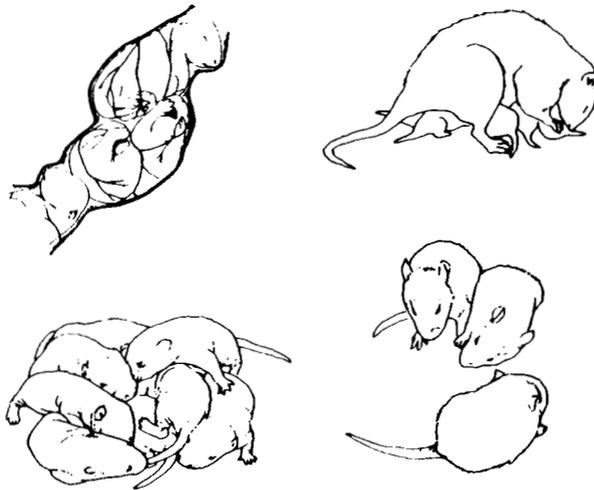


Figure 1. Four Ontogenetic Habitats in early development of the Norway rat. (A) The Uterine Habitat; (B) Maternal behavior as Habitat (C) Huddle as Habitat; (D) the Coterie as Habitat. This sequence is typical in mammalian development. (From Alberts and Cramer, 1988.)

Lighting requirements in microgravity is an environmental issue, concerned specifically with the quantity, quality, and periodicity of luminance. Mammalian development can also be

characterized in environmental terms. Alberts and Cramer (1987) recently argued that all developing mammals experience during early ontogeny an invariant sequence of environments, which we termed "ontogenetic habitats" that are remarkably similar across diverse species. Figure 1 depicts four early ontogenetic habitats for the Norway rat.

The four habitats illustrated in Figure 1 are: The Uterus as Habitat; the Mother as Habitat; the Huddle as Habitat, and the Coterie as Habitat. All rats experience this sequence during development. Each of these stations in life have varied and specific environmental characteristics.

THE UTERUS AS HABITAT

Life, including behavioral life, begins before birth. In all mammals, the uterus is the first environment to which the fetus displays behavioral and physiological adaptations. The uterine habitat is an aqueous world in which gas and nutrient transfers are carried by the umbilical cord. The fetus is connected to the mother's body at the placenta via this flexible conduit and tether. In the uterine habitat, littermate siblings reside in close proximity to one another (see Figure 1) although each is encased in its own, fluid-filled amniotic sac. Respiratory movements begin early, just 16 days after conception. The total gestation period of the rat is about 21.5 days.

Behavioral Embryology. The fetus' early respiratory movements are shallow and irregular. Of course, there is no free air to respire in the uterine habitat, but these fetal respiratory movements are sufficient to move fluids through the

developing nasal cavity and trachea. Fetal respiratory movements are considered important preparation for successful adaptation to the postnatal habitats where oxygen exchange across the lung surface is required. Prenatal conditions in which fetal respiratory movements are blocked or dampened are associated with diminished lung volume and the inability to breath adequately after birth (Liggins, 1982).

Swallowing is another fetal behavior. Fetuses swallow significant quantities of amniotic fluid. Smotherman and Robinson (1985) have discussed the role of fetal swallowing in terms of ingestion of amniotic fluid. This activity is presumed to serve at least two functions in the life and development of the late-term fetus. Ingestion of amniotic fluid permits digestion and excretion, thus stimulating the entire gastrointestinal system.

Although the uterus is an aqueous environment, the fetus does not float in it. In the uterine habitat, the fetuses are bound rather tightly by the confines of the amniotic sacs and the uterine walls. Fetal swallowing aids in the clearance of amniotic fluid which changes in composition and consistency as gestation progresses. During gestation the amniotic fluid thickens and, rather than serving as a medium for fetal buoyancy, it appears to act as a lubricant, facilitating movements.

Like all vertebrate fetuses, the prenatal rat displays head flexures, which expand to encompass more of the anterior trunk and eventually recruit and involve limb movements (Bekoff, 1985). Interlimb coordination follows a definable, developmental

sequence. Detailed observations of fetal behavior have revealed other early-developing coordinated movements, including mouthing (Smotherman and Robinson, 1985).

Fetal movements appear to be important. Pharmacological blockade of fetal movements can lead to fused joints and impaired bone and muscle formation. Clearly, factors that stimulate and maintain fetal movement are important in development and can have significant longterm consequences.

Fetal Experiences and Cyclicity. What kinds of stimulation might be experienced by the rat fetus? The answer to this question depends largely on the status of the fetus' sensory systems, a topic with many unknowns. From the available data, however, we can assert that the rat fetus probably detects (experiences) changes in temperature, vestibular stimulation, cutaneous as well as chemical cues detected via its senses of smell and possibly taste.

Though much remains to be learned about fetal life, we already have learned to appreciate that the fetus is an active, behaving creature. Pertinent to the topic of the present essay, the fetus receives a variety of forms of cyclic stimulation, and itself exhibits cyclicity.

The mother's behavior and physiology are potential sources of fetal stimulation that can affect the fetus. There exist circadian rhythms to maternal activity, body temperature, feeding, drinking, and self-grooming (which could provide tactile stimulation). But, what can a fetus detect and does it have the capability to be entrained by such stimulation? We can recognize several possible avenues of maternal entrainment of fetal

activities. The behavior of the pregnant dam has numerous circadian aspects (as does that of the nonreproductive rat) and it is possible to trace real and hypothetical pathways of influence from light-entrained features of the mother's world, to that of her unborn young.

The profile of thermal, metabolic, biochemical, and endocrine events that underlie the mother's circadian patterns of locomotor activity, feeding and drinking, sleeping, body temperature, and social behavior can be traced through real and hypothetical pathways of influence from light-entrained features of the mother's world to that of her young.

Fetal Oscillations. Fetal brains have active, circadian pacemaker cells in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. The SCN regulates rhythmic expression of behavioral and physiological processes in mammals. In adults, the SCN is entrained to 24-hr day by the light-dark cycle, which reaches the SCN via a monosynaptic retinohypothalamic pathway.

Reppert and his associates (e.g., Reppert, Henshaw, Schwartz and Weaver, 1987; this volume) have used 2-deoxyglucose autoradiography to measure circadian rhythms in fetal SCN. They found circadian activity in fetal SCN during late prenatal life, before the retinohypothalamic connection is established with the SCN. It currently appears that the maternal circadian system entrains but does not drive the oscillations of fetal SCN. It has been suggested that in rats, maternal entrainment of fetal SCN activity may begin as early as Day 10 of gestation (Honma, Honma, Shirakawa & Hiroshige, 1984a,b).

Lesions of the mother's SCN on Day 7 of gestation abolishes the normal profile of circadian development in the fetuses and even in postnatal pups. The effect seems to be based on desynchronization rather than elimination of oscillations. For instance, pups born to SCN lesioned dams and reared in constant darkness, later showed circadian rhythms in ingestion, but they were not synchronized, as were similarly reared pups born to mothers with an intact SCN (Reppert & Schwartz, 1986).

There is little detailed understanding of the functional significance of fetal circadian rhythms. Nevertheless, pervasive and important coordinative and maintenance roles in later physiological and behavioral processes make reasonable most expectations that these rhythms will be shown to have important developmental roles. Recently, it has been suggested that fetal rhythms may contribute directly to the timing of birth in mammals (Reppert et al., 1987). Thus, in the design and evaluation of habitats for both reproductive and developing animals, we should remain aware of the subtle interactive forces at work in these living systems.

Maternal Stress and Fetal Development. There are other maternal effects on fetal development, including those associated with non-specific forms of stress. One relevant example can be derived from a spaceflight experiment. The flight of the Soviet biosatellite, Cosmos 1514 carried 10 pregnant rats during its 5-day flight (encompassing days E12-E17 of the normal, 21-day gestation). Male rats born to Flight mothers had shorter anogenital distances. The AG distance is a morphological correlate of masculinization or sexual differentiation in the

male. Maternal stress has been shown to feminize male fetuses and is evidenced at birth by the decreased AG distances. It is crucial to recognize that the same effect was observed in the males born to dams in the non-flight, Synchronous control group. This indicates that the feminization effect was not related to the microgravity conditions of space but correlates with other common factors such as the housing, diet, vibrations, and other disruptive effects of the flight habitat (Alberts, Serova, Keefe and Apanasenko, 1986).

Though the feminization of the male fetuses was not a flight effect, it serves to illustrate the kinds of broad, integrative effects that can be exerted on the fetus by environmental events. The unique and specialized relationship between the uterine environment and the world in which the mother resides means that the fetus is susceptible to at least some of the mother's experiences.

MATERNAL BEHAVIOR AS HABITAT

The event of birth marks an irreversible change of address in the development of every mammal. This momentous occasion is probably unexcelled as an adaptive challenge. The fetus moves from an aqueous environment inside the mother's body, where life support is managed largely via the umbilicus, to a gaseous environment in which life support is a far more complex affair. Nutrients must be actively sought and extracted from the mother's teats. Body temperature regulation also becomes a more active enterprise (Alberts, 1978a; Hull, 1973).

Rats are born in litters; they leave the uterine habitat

sequentially and take up residence with their littermates in a natal nest. In this nest, the mother is a salient feature. Indeed, in our schema of mammalian ontogenetic niches, the mother and her behavior are noted as the next niche.

Maternal Behavior Cycle. Maternal behavior in mammals, including rats, has been amply documented and described (e.g., Weisner & Sheard, 1933; Rosenblatt & Lehrman, 1963; Gubernick & Klopfer, 1981). Typically, maternal behavior is separated into a profile of common, representative activities. For the Norway rat these usually consist of nursing, licking, nest-building, and transport. These behaviors can be directed at the litter as a whole (e.g., nursing) or at individual pups (e.g., licking).

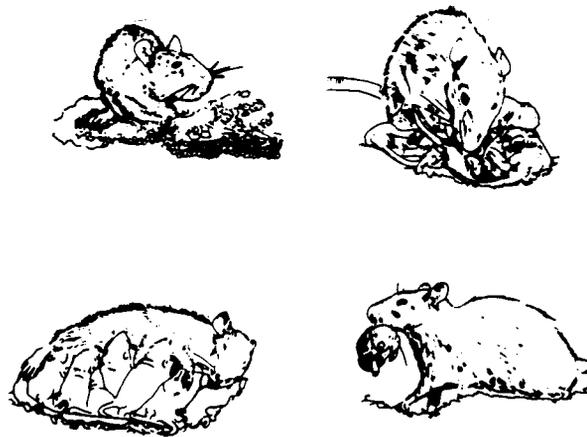


Figure 2. Maternal behavior of the Norway rat, like that of many mammals, consists of defineable, quantifiable activities such as (A) nest-building, (B) licking the offspring, (C) nursing, and (D) transport. (From Alberts and Gubernick, 1983.)

The temporal organization of maternal behavior is highly patterned. The mother approaches and contacts her litter in the natal nest. While there, she engages in a melange of maternal activities. These nest bouts are separated by her departures, during which she forages, feeds, grooms, explores, rests, etc.

Thus, we can consider the rhythmicity of maternal visitations (nest bouts) and, in so doing, implicitly recognize the rhythmicity of the various activities subsumed under the inclusive category of maternal behavior.

Maternal behavior in the rat is expressed with a clear circadian rhythmicity. Figure 3 shows an idealized picture of the circadian aspects of maternal behavior. The solid line depicts the maternal presence in the nest across the maternal behavior cycle, lasting from the birth of the litter to the age at which mother and pups separate during the course of weaning.

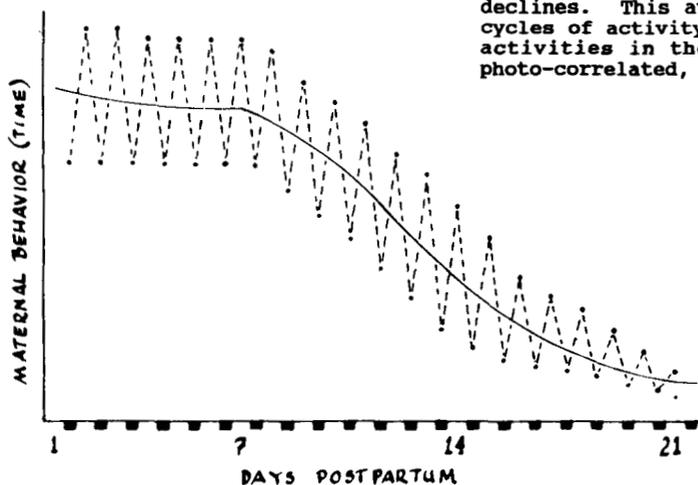


Figure 3. Cycle of maternal behavior in the rat, including circadian aspects. The solid line depicts the general course of maternal behavior throughout the lactational cycle. Maternal behavior (e.g., nesting, licking, nursing, etc.) is exhibited at high average rates during the early postpartum period and then declines. This average profile, however, is derived from daily cycles of activity, depicted by the broken line. Such circadian activities in the dam's behavior provide the offspring with photo-correlated, circadian patterns of stimulation.

The frequency and duration of maternal visitations to the nest decline progressively after an initially high and intense maintenance phase. Nevertheless, as the broken line on the graph shows, there is built-in a circadian pattern of visitation. Maternal presence is most frequent during the light hours. Lactating rats tend to spend more time away from their litters during the dark hours, when adult rats typically are most active.

Thus, the mother feeds predominantly during the dark hours and nurses during the light. Ader and Grotta (1970) maintained light-entrained rats under constant photic conditions and continuous circadian periodicity of maternal behavior, which indicated internal pacemaker control, either direct or indirect, of maternal behavior pattern.

Maternal Stimulation. From the litter's point of view, visitation means stimulation -- many kinds. When the mother joins the litter in the nest, her brooding contact provides thermal stimulation. The mother's body is consistently warmer than that of her offspring. This is a mammalian trait. Neonatal mammals are thermally fragile; they lose body heat more rapidly than their adult or older counterparts and are typically in thermal jeopardy without some form of thermal protection, such as that derived from brooding, an insulated nest, swaddling, even huddling with others. There have been a number of detailed studies of the thermal relationship between a mother rat and her litter. Heat balance is an important determinant of mother-litter interactions (e.g., Leon, Croskerry & Smith, 1978).

When the mother joins her litter in the nest, she is often observed to lick each pup individually. Such licking is often focussed at the pups' anogenital (AG) regions. This form of tactile stimulation triggers the pups' micturition reflex and they void. The mother avidly consumes the urine, thereby reclaiming about 2/3 of her lactational water. The pups, in turn, are voided, cleaned, aroused, warmed and cooled, and usually then attach to a nipple (Alberts & Gubernick, 1983;

Gubernick & Alberts, 1983).

It has been suggested that in rats, another consequence of this anogenital licking is masculinization of the recipient. Indeed, male pups receive about three times more anogenital licking than do their female littermates (Moore & Morelli, 1981)! The gender difference in AG licking is related to an androgen-dependent substance in the male pups' urine that the mother finds especially attractive. Experimental manipulations suggest that the amount of licking received during infancy can affect gender-specific aspects of copulatory behavior during adulthood.

There are important practical implications from this curious, gender-related phenomenon. Factors that disrupt the patterning of maternal behavior will inevitably affect the expression of multitudes of developmentally-significant stimuli. Lighting is an important determinant of maternal behavior and there are numerous potential developmental effects, both direct and indirect.

THE HUDDLE AS HABITAT

After the first postnatal week, the mother spends more time away from the nest. She remains attentive to the pups and continues to be their sole source of nutrition, but her excursions become more frequent and longer. In the absence of the dam, the litter remains organized in a coherent clump, or huddle. Huddling behavior is an active, regulatory behavior. It affords the pups a significant thermoregulatory advantage and conserves valuable metabolic energy (Alberts, 1978a). Together, the pups in the litter aggregate exhibit a form of "group

regulatory behavior" whereby the huddle regulates its configuration so that the surface area of the group increases and decreases as a function of the ambient temperature. Group regulatory behavior changes is accomplished by nearly continuous activity and adjustments by pups in the group, illustrated in Figure 4 (Alberts, 1978a). Thus, in the absence of the dam, this type of activity is triggered, indicating that the circadian pattern of maternal behavior is temporally complemented by group regulatory activities of the pups (Addison & Alberts, 1980).

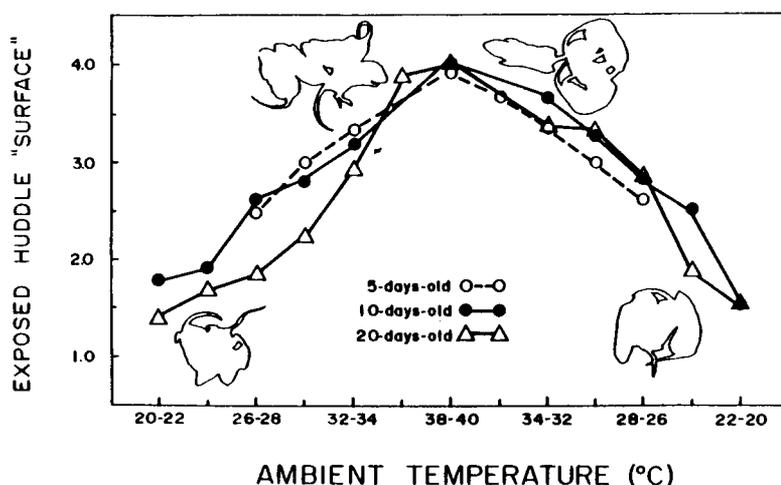


Figure 4. Group regulatory behavior by huddles of rat pups. The graph depicts temperature-dependent regulation of huddle "surface", which has been shown to be vital in the pups' temperature regulation and conservation of metabolic energy (From Alberts, 1978a.)

Sensory and Perceptual Development. The time during which pups live more or less exclusively in the Huddle habitat (postnatal Days 0 - 20), is a period of rapid and dramatic maturation. Sensory development, for example, proceeds quickly, within and between sensory systems.

The pups' sense of smell is functional at birth and, for some vital behaviors such as nursing olfaction is necessary. Nevertheless, olfactory acuity and discrimination improve

considerably over the first three weeks postpartum (see Alberts, 1984).

Beginning about Day 12, the pups' ears unseal, and there is an immediate improvement in auditory sensitivity associated, no doubt with the removal of a mechanical and fluid buffer between the tympanic membrane and the acoustic environment. Even after ear-opening, however, auditory perception develops considerably. Generally speaking, auditory sensitivity during development progresses from low to high frequencies (see Alberts, 1984 for review). Figure 5 depicts some representative data on development of acoustic sensitivity in rats. The "tuning curves" illustrate the animals' range of greatest acuity.

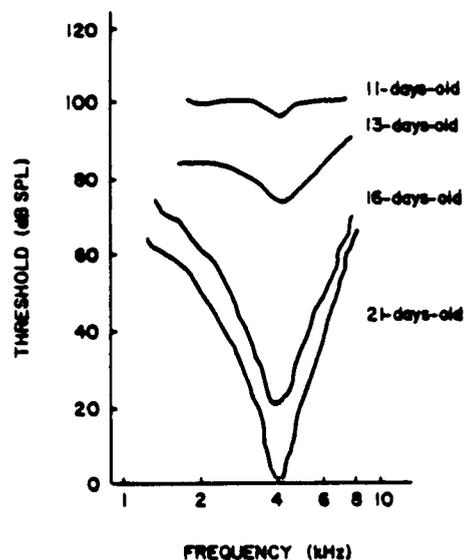


Figure 5. Frequency-intensity coordinates for tones that elicit discharges just above non-stimulated levels from single units in rodent brainstem. These tuning curves were centered at 4kHz to illustrate the age-related decrease in minimal threshold seen from Days 11 to 21. (Redrawn from Clopton, 1981.)

It is notable that rat pups emit high frequency vocalizations (22 - 40 kHz) sometimes called "ultrasounds" (but this is an anthropomorphic term, because such high frequency cries, while beyond the range of the human ear are readily perceived by adult rats).

Pups appear to respond to acoustic cues from other rats (e.g., Alberts and Leimbach, 1980). I know of no systematic studies of the roles of auditory cues in the early life of developing rats, so I can only postulate about "acoustic zeitgebers" in the pups' environment. It would be prudent to include such considerations in the design of animal habitats in space, at least to the extent of being aware of any circadian or periodic changes in the acoustic environment, for this might serve to entrain the animals, even at early ages. In addition, until more is known about the roles of social sounds in their lives, habitats should be carefully evaluated, to avoid inadvertent masking of socially-important acoustic cues. For instance, florescent light sources can be noisy. Sources of illumination can thus create acoustic stimulation correlated perfectly with an animal's light cycle. Engineers and researchers should be aware of such possibilities in evaluating flight habitats used for studies of rhythmicity in rodent development.

Vision is the final sensory system to become functional in vertebrates (Gottlieb, 1971). The rat pups' eyelids unseal on Day 14 or 15, at a stage when they spend most of their time in the huddle. In laboratory environments, with relatively bright, overhead lighting (see Williams, this volume), avoidance of light may be part of the pups huddling response (Alberts, 1978b). Prior to eye-opening, the pups' can detect light through their unopened lids, but there have been no studies of the behavioral significance of such photic stimulation (for reviews, see Alberts, 1984; Gottlieb, 1971).

As soon as pups become photosensitive, they are probably entrainable to photic stimulation. It is essential to include multiple measures in assessments of onset of entrainment to photic stimulation, because not all circadian systems mature simultaneously. Furthermore, it is possible that some systems may be more susceptible to social influences such as that from the mother, than to luminance. Generally speaking, past research with rodents has overlooked measurement and control of light wavelength and intensity. The significance of these basic parameters in development is poorly understood.

Nearly all interpretation of light perception in developing rodents is concerned with visual (retinal) detection. There exist some reports, albeit controversial, that the rat pup may also possess an extraretinal phototransducer. One hypothesized candidate is the Harderian gland, which is a porphyrin-containing structure located behind and around the eye. There are reports that the circadian rhythm of pineal serotonin is abolished by removal of the Harderian gland in blinded animals (e.g., Wetterberg, Geller, and Yuwiler, 1970). The significance of these claims remains to alert us to the possibility that there are important, undiscovered avenues of perception. These have yet to be definitively established.

Social Development. The Huddle habitat is also the site of considerable social development. In the huddle, pups are stimulated by combinations of cues that have been shown to work together to reinforce and shape perceptual preferences, social attachments, and to regulate behavioral state and physiological responsivity.

The role of light and of other non-photic forms of cyclic stimulation may be important contributors to the maintenance and coordination of physical and social interactions. These can be identified and analyzed on the basis of existing knowledge. For our present purposes, it is appropriate to recognize these factors and note that proper habitat design would preserve these important forms of environmental support and behavioral organization.

Endogenous Activity in the Huddle? In past research, litter aggregates have been analyzed as if they were single, unitary organisms (Alberts, 1978a; Mount, 1960). Indeed, huddles of littermates do show remarkable group activities. For instance, underlying the "group regulatory behavior", described earlier (see Figure 4, above), is a nearly continuous, active rooting and burrowing amid littermate bodies.



Figure 6. Several views of a huddle of 10-day-old rats depicting the flow of bodies through the group. (From Alberts 1978a.)

Alberts (1978a) described and quantified this "pup flow",

which is illustrated in Figure 6. Pup flow through the huddle is regulated by nest temperature and therefore varies in relation to maternal behavior (Addison and Alberts, 1980). The mother's heat-providing presence in the nest is circadian (Leon et al., 1978). Thus, although it has not been shown that the huddles activity is cyclic, it correlates with the dam's cyclic, photoperiodic behavior and thus might well be a behavior indirectly driven by photic cues, via the mother's behavior.

THE COTERIE AS HABITAT

Entry into the rat pups, next ontogenetic niche seems to be dictated by the pups' increased sensory and motor capabilities. During the 3rd week postpartum, the pups' eyes and ears unseal. Audition and vision are primarily "distal" senses that complement the pups' olfactory, tactile, and gustatory experiences. Combined with locomotor competence, speed and increased endurance, the pups leave the nest and diminish their reliance with the mother's body. Weaning begins. During this phase, characterized by the eventual achievement of independence from the natal nest, the pup's behavior is dictated by social cues. The social group (characteristic of the colonial life of the Norway rat) becomes the niche: this is the Coterie as habitat.

In the Coterie habitat, social stimulation is a potent force. Social behavior among juvenile rats includes play, mutual grooming, resting and sleeping in groups. The presence of another rat, particularly an adult, affects the onset of feeding - a landmark event in the maturation of every mammal - as well as the very substances that the pup recognizes as acceptable foods

(Galef, 1977). The appearance of novel forms of behavior can be stimulated by social conditions (Galef, 1982).

While living in the Coterie habitat the pups make a dramatic shift in their circadian pattern of energy intake. Pups get mothers milk primarily during the the light phase of the L/D cycle. During the dark phase, the mother engages in foraging and eats. Pups display an identical tendency to feed during the dark hours. Thus, during weaning, the transition from milk, consumed during the day, is replaced by food, which is consumed during the night (Thiels and Alberts, 1985).

Generally speaking, there is continued developmental entrainment in circadian rhythmicity throughout life in the coterie. Circadian rhythms in locomotor activity, sleep and waking cycles, neurochemical systems of norepinephrine and serotonin, and physiological oscillations body temperature such as the dark-phase surges in plasma corticosterone do not match adult patterns until roughly Days 35-40. This age range encompasses weaning and the gradual achievement of independence from the natal nest. It is a phase of rapid growth, continued differentiation, and is a prelude to puberty.

Kittrell and Satinoff (1986) implanted biotelemetry thermistors in the bodies of rat pups 10-15 days of age and monitored them for the next two months in order to establish the development of the circadian temperature rhythm (CTR). The rats lived in a 12hr light/ 12hr dark photoperiod. These investigators concluded that the CTR develops after pups' other thermoregulatory mechanisms achieve adultlike parameters, suggesting that the controls of the CTR are functionally

separable from other thermoregulatory controls in the pups. From Days 25 to 45 the pups' dark period body temperatures were more frequently higher than their daily mean. By Day 42, the adult pattern in CTR was seen: 90% of body temperature reading during the dark phase were greater than the daily mean whereas the light phase measures were below the daily mean.

Kittrell and Satinoff (1986) emphasize that the development of the CTR does not limit the pups' ability to make significant thermoregulatory responses to environmental challenges. They view the CTR as an important component to the pups' adaptation to the post-weaning environment.

SUMMARY REMARKS

Cyclic activity in suprachiasmatic nucleus is seen prenatally. Fetal circadian rhythms can be entrained by the mother, via unidentified, behavioral or physiological pathways. After birth, the pups' circadian activities can be further entrained by maternal behavior. The mothers presence in the nest, and hence her nursing, licking, brooding behaviors, are definitively circadian in organization. Maternal presence is greatest during the light phase. Although prenatal entrainment is probably in place, maternal behavior and stimulation further entrain the offspring. Conditions in the postnatal habitats can maintain or modify prenatally established rhythms. It is most likely that photic effects on the newborn are indirect, i.e., via influences such as maternal behavior, because the pups' visual system remains highly rudimentary.

During the normal course of the lactational cycle, maternal

presence in the nest is gradually withdrawn, leading to the emergence of the Huddle as an ontogenetic habitat. In the Huddle there persist circadian patterns established by the dam's behavior. Thus, the mother maintains the entrainment with her periodic, circadian visits. In the Huddle, important thermoregulatory and behavioral interactions among the pups are conducted. These are punctuated by the dam's appearance and the delivery of milk and other critical forms of maternal stimulation. Sensory maturation is rather profound during this period. Although the pups' eyes are closed until about Day 15, it is likely that environmental illumination (particularly in a typical laboratory setting) can be perceived through the sealed eyelids. (See Williams, this volume.)

By third postnatal week, the rat pup is operating with a complete set of sensory systems and is capable of running, climbing, and jumping. Weaning begins, and the huddle and maternal behavior become increasingly occasional habitats, as the larger, social milieu or Coterie takes over as the next ontogenetic habitat. Under the coterie's influence, food selection begins and social activities become shaped. The delicate and critical events are dependent on interactions coordinated in time, so group entrainment is a necessary element.

MAMMALIAN DEVELOPMENT AND REPRODUCTION IN MICROGRAVITY

Two Soviet satellites have provided the only glimpses at mammalian development in microgravity. In 1979, three male and seven female rats orbited for 17 days. Figure 7 is a drawing of the Bios, or flight cage used on several Cosmos missions. The

partition separating the sexes had been lifted 3-days after launch, so the mixed group mingled for two-weeks in microgravity. With a 4-5 day estrous cycle, it seemed reasonable to expect that the rats would have two or three chances to mate in microgravity and give mammalian development its very first chance to proceed in the absence of gravity. It appears, however, that there were no pregnancies derived from the flight.

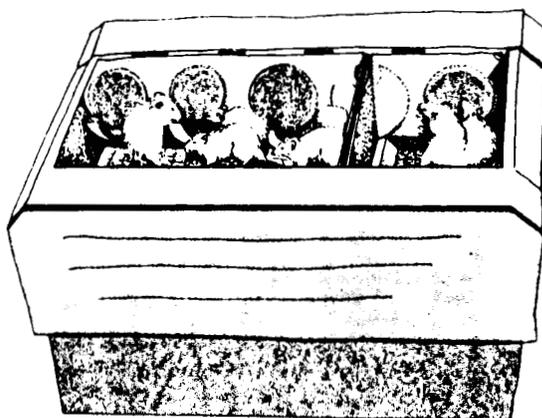


Figure 7. Drawing of the Soviet's Bios compartment used to fly groups of adult rats in their Cosmos Biosatellite. The cage depicted in this drawing carried the unsuccessful mating experiment on Cosmos-1129, and the successful flight of pregnant dams on Cosmos-1514. (9)

There are reasons to expect that rats would have great difficulty mating in a large, rather voluminous habitat such as the Bios (discussed in detail by Alberts, 1983), but such issues appear secondary since the ground controls also failed to produce litters! It has been hypothesized that there may have been a light leak into the rat compartment from an adjacent section of the satellite (Keefe, personal communication, see Appendix D in Alberts, 1983). This may have altered the rats' luminance period sufficiently to disrupt the females' estrous cycles and hence prevent the possibility of mating and fertilization. The possibility that lighting perturbations may have interfered with

the necessary reproductive precursor to mammalian development is a poignant indicator that lighting requirements for space research is a fundamental topic.

In December, 1983 the Soviets again launched an unmanned Cosmos biosatellite. Cosmos-1514 contained the next attempt to evaluate mammalian developmental processes in space. This time the rats were bred on earth and were launched on the 14th day of gestation, a point at which implantation has had plenty of time to occur. It was a relatively short orbital flight, 5-days-long, so the pregnant rats were recovered about 3 days before the time of their normal parturition. Figure 8 puts the microgravity exposure in a chronology of developmental landmarks relevant to neural and behavioral development.

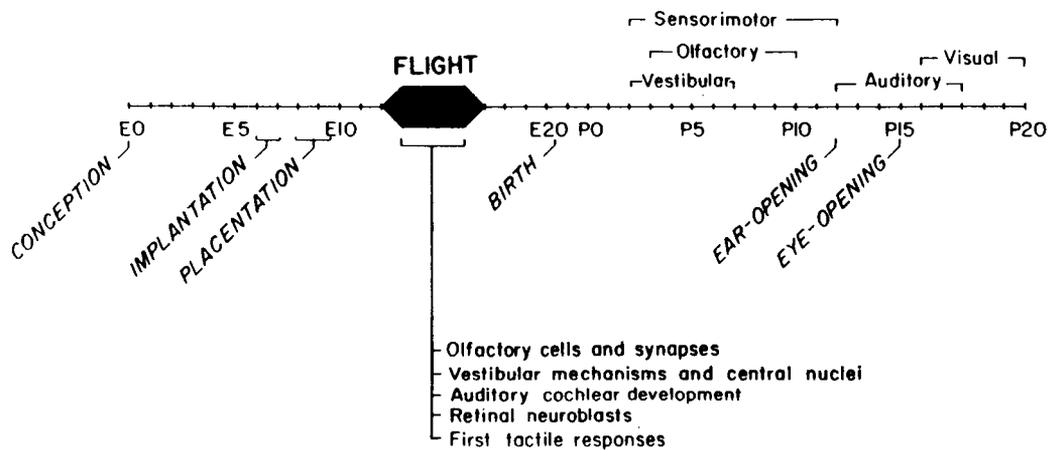


Figure 8. Some developmental milestones in rat development from conception to postnatal Day 20, featuring events in sensory development. The shaded area indicates the occurrence of the microgravity exposure provided by Cosmos-1514. Postnatal sensory tests were conducted on the offspring.

Five of the 10 Flight dams were sacrificed at recovery. From each uterine horn was taken at least one fetus for neuroanatomical assessment. The brains of the flight fetuses displayed several features that could be considered abnormalities

in relation to the gestational age (Keefe, et al., 1986).

The second group of females were transported from the recovery site to the Moscow laboratory. They were permitted to go to term and, indeed, four of the five rats gave birth to full litters. The remaining dam had a prolonged labor and eventually delivered normally-formed, nonviable pups. The difficulty was attributed to blockade of the birth canal by one pup that had suffered an apparent hematoma and whose head was enlarged. Postnatal testing of the live pups from the other four Flight litters began on Day 1. We administered a battery of sensory and motor tests designed to establish the functional patency of the tactile, vestibular, olfactory, auditory and visual systems. In addition, these tests and other observations included systematic video records of the pups' movements and, through these, we later studied the development of coordinated, adaptive movements in the infants (Alberts, Serova, Keefe & Apanasenko, 1986).

Flight pups performed as well as the control rats in the postnatal tests. In this sample of pups there were numerous observations pointing toward interesting trends for future studies, but the major outcome of Cosmos 1514 was that mammalian development sustained this first-time challenge of microgravity. Obviously, it is essential to expand into broader microgravity challenges at points and stages of development and for longer periods.

The requirements of this preliminary study of development in space did not permit basic manipulations for studying circadian rhythmicity, such as maintenance of animals under constant luminance conditions. Rats were housed in a 16:8 LD cycle before,

during and after the flight. We nevertheless noted a couple of relevant aspects of periodicity in our observations. One was the circadian pattern in the rat's maternal behavior.

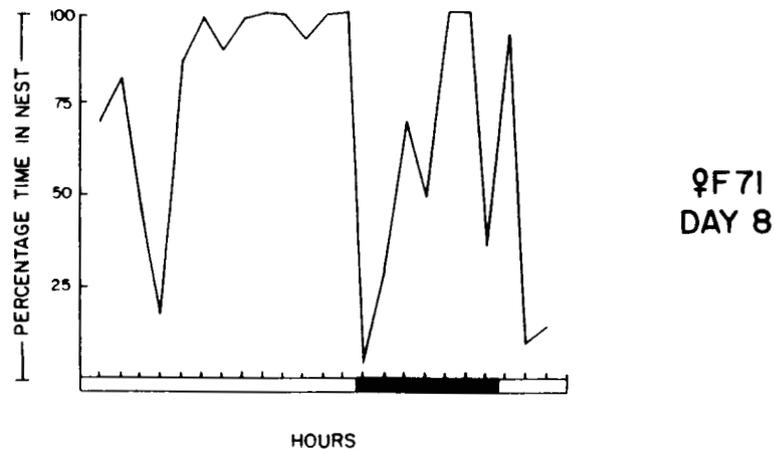


Figure 9. Circadian aspects of nest visitations and maternal behavior in a Flight female from Cosmos-1514. This normal pattern of maternal behavior was maintained throughout the post-flight, postpartum period. Animals were maintained throughout a cycling, light-dark schedule. Mammalian rhythms have not yet been studied under constant illumination or darkness in space.

Figure 9 shows the amount of time spent by a Flight dam in the nest with her pups on Day 8 postpartum. The dark bar along the horizontal axis shows the dark hours of the day. This representative mother displays the typical circadian pattern of maternal nest visitation. Unfortunately, these incidental observations are insufficient to make even preliminary remarks concerning the role of lighting or circadian rhythms on mammalian development in space. The paucity of available data emphasizes the need to undertake systematic studies in this important area of biological and biomedical interest.

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PHOTOIMMUNOLOGY

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INTRODUCTION

Photoimmunology is the study of the effects of nonionizing radiation on immunity. It is a relatively new branch of biologic research only having attracted much interest over the past decade. Before discussing this subject, I will first introduce a few terms that have not been introduced by previous speakers. Ultraviolet radiation is divided into three wavebands: Ultraviolet A or UVA (320 to 400 nm), Ultraviolet B or UVB (280 to 320 nm) and Ultraviolet C or UVC (200 to 280 nm). Most study in photoimmunology has concentrated on the effects of UVB radiation emitted by Sunlamp bulbs and that tends to be used synonymously with UV radiation by most workers in the field. Almost all work that I discuss has been conducted on C3H mice, a pigmented strain, and BALB/c, an albino strain. There are some dosage variations between these two strains but they are not of great significance. All effects so far described are mediated through the skin and not, so far as is known, through the eyes. Both local and systemic alterations have been reported. The term "local" effects refers to those observed and elicited at the site of exposure to radiation. Systemic effects are those seen at a distant non-irradiated site.

Photoimmunologic alterations have been found at molecular, cellular and immune response levels and I will be concentrating on the latter: changes in immune responses observed in the intact organism. I propose to concentrate on two: suppression of rejection on UV-induced tumors and suppression of contact hypersensitivity (CHS). The reason for this focus is that they provide good examples of how photons can influence immune function and secondly, we understand more about these effects than any others. An important feature of the suppressive effects of photons is its selectivity. An animal exposed to non-ionizing radiation does not exhibit pan-immunosuppression, as is seen with ionizing radiation and many immunosuppressant drugs, but there might be suppression of CHS while antibody responses remain intact. This feature of selectivity may be a great advantage, particularly from the point-of-view of using nonionizing radiation for the therapeutic manipulation of the immune system.

SUPPRESSION OF TUMOR REJECTION

Much work in photoimmunology has focused on how and why UV radiation is able to influence the growth of UV-induced tumors through suppression of normal immune function. Dr. Margaret Kripke, now at the M.D. Anderson Hospital and Tumor Institute in Houston, has been responsible for initiating and conducting much of the work in this area and this work has been recently reviewed (1).

Exposure of mice to UVB radiation results in the development of tumors at the site of exposure, and in C3H mice most animals have developed tumors by 40-60 weeks. The tumors are almost all squamous cell carcinomas (2) which is also the most important sun-induced, nonmelanoma skin cancer in humans. The initial observation that triggered interest in the immunobiology of these tumors was that the tumors did not grow following transplantation into normal syngenic

mice but did grow in immunosuppressed mice. The reason for this is that the tumors are highly antigenic and in the normal mouse they are rejected by a T cell-mediated immune response. This observation raised the question of why the tumors were able to develop in the original host. The answer is that UV radiation produces selective suppression of the normal immune rejection response by stimulating the generation of suppressor T lymphocytes. The dose of radiation required to produce suppression is small and far below a carcinogenic dose. Fisher and Kripke have recently shown that the generation of suppressor cells plays a central role in the development of UV-induced skin cancer (3). Thus UV radiation produces in mice at least two alterations that are involved in the development of skin cancer: neoplastic transformation of cells and selective immune suppression permitting expression of those cells as tumors.

PHOTOIMMUNOLOGY AND CONTACT HYPERSENSITIVITY

CHS is another immune response that can be altered by exposure to nonionizing radiation and, in most instances, the alteration is in the direction of suppression. Two systems have been used to study UV-induced suppression of CHS: local suppression and systemic suppression.

In local suppression of CHS in mice, the hapten is applied at the site of exposure to radiation, for example the back, and then the challenge dose of hapten is applied a few days later to the ears, which have been kept covered during the exposure to radiation. The amount of ear swelling is taken as a measure of the degree of the CHS response. Mice exposed to a low dose of UVB radiation exhibit a suppression of the response, usually of the order of 70-90% reduction. The suppressed response is associated with the generation of hapten-specific T suppressor cells (4). Much work has focused in the alterations that initiate the generation of these cells with particular emphasis in changes in the skin at the site of exposure to radiation. Langerhans cells, an antigen-presenting cell (APC) in the epidermis, are structurally and functionally altered by exposure to similar doses of UV radiation (5). It is thought that UV-induced alterations in antigen presentation results in preferential activation of suppressor rather than helper cells.

Systemic suppression of CHS involves a somewhat different model. Mice are exposed to UV radiation on the shaved back while the ears are covered, for example, by black electrical tape. The hapten is applied to the belly of the mouse, which was not exposed to radiation, and a challenge dose is applied a few days later to the ears. Thus, both the site of induction and elicitation of this immune response are not exposed to radiation and, therefore, any effect must be systemic in nature. Exposure to a high dose of UVB radiation, of the order of 10 J/m^2 , produces about an 80% reduction in the CHS response. This suppression is also associated with generation of hapten-specific T suppressor cells (6). Green found that UVB radiation caused a suppression of APC function in the spleen and postulated that this led to preferential activation of suppression rather than help (7). Kripke and I have been investigating this possibility recently along with other potential explanations. We have found that the splenic APC defect is not an essential element in the pathway to suppression and nor are changes in Langerhans cells in the skin of circulating mononuclear cells (8-10). Swartz has found that a low molecular weight substance is present in the serum of irradiated mice which can initiate the pathway to suppression of CHS (11). Swartz, in Vienna, has found that

epidermal cells in culture produce a factor following exposure to UV radiation which can also produce suppression of CHS in vivo (12). Thus, a substance produced in irradiated skin and released into the circulation possibly initiated the suppression of CHS but the site of action of this substance is unknown.

Systemic suppression of CHS is not confined to the mouse. Exposure of strain 2 guinea pigs to UVB radiation produces suppression of CHS with appearance of T cells that act on the induction phase of the immune response (13).

The action spectrum for systemic suppression of CHS has not been determined and wavelengths around 260-270nm appear to be most effective. This finding has led to speculation as to the nature of the chromophore with two, urocanic acid (14,15) and DNA (16) being suggested possibilities. Other wavebands of radiation have also been studied for their effect on CHS in the mouse. A 72-hour continuous exposure to UVA radiation, with all wavelengths shorter than 315nm filtered out, produces some enhancement of CHS. Under similar conditions of exposure, visible radiation (>400nm) from weathered mylar filtered coolwhite bulbs produces a small but consistent suppression of CHS. Control mice for these experiments (17) were kept under conditions of constant lighting to eliminate the influence of altered circadian rhythms. These treatments have quite marked effects on the skin at the site of exposure with hyperplasia of the epidermis and structural alterations in Langerhans cells.

Finally, sunlight has also been used as a source of nonionizing radiation in some experiments (17,18). A three day exposure of both mice and guinea pigs to summer sun produced systemic suppression of CHS and this effect was abrogated by a mylar filter or application of a sunscreen; the latter finding suggests that wavelengths between 300 and 315nm are responsible for the effect. Sunlight also induced suppression of rejection of UV-induced tumors in mice and again, filtering indicated that wavelengths of approximately 315nm were responsible for this effect (18). Sunlight, of course, is a very complex light source and any of its effects are probably not just the sum of the effects of its individual wavelengths. An additional complicating factor is that the effects we observe from exposure to nonionizing radiation may be in part or whole due to interaction with exogenous photosensitizers present in skin. For example, we found that psoralens in combination with UVA radiation can induce suppression of CHS (19). Psoralens, of course, are naturally occurring photosensitizers present in many fruits and vegetables and the presence of such substances can alter or enhance the effect of radiation alone.

LEON: Could you use this phenomenon to advantage in facilitating organ transplantation?

MORISON: Selective manipulation of immune function may have considerable therapeutic potential in tissue and organ transplantation and has already attracted some study. We have shown that graft-versus-host disease in skin following bone marrow transplantation can be prevented by UVB treatment in mice (20) and can be treated by exposure to psoralen and UVA radiation in humans (21). In addition, exposure of pancreatic islet grafts to UV radiation prevents their subsequent rejection in rats (22) and this has potential in the treatment of diabetes.

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LIGHT EFFECTS ON ANIMAL LEARNING, MEMORY, AND PERFORMANCE

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Light and the lighting environment can have important effects on learning, memory and performance, the plastic systems that enable an animal to change its behavior in response to a changing environment. Since microgravity effects on learning and performance are of considerable practical and theoretical interest, discussions of lighting design for future microgravity habitats for animals should involve some consideration of the effects of lighting on these plastic systems. For the purposes of this discussion, we can divide light effects into 3 types. First, light has a critical direct parametric role in visual tasks. Second, light can exert a powerful contextual influence on learning, memory, and performance, and third, there are indirect effects of light that can influence performance through several other mechanisms.

In visual tasks, light is a specific part of the stimulus complex that controls the task, either in the form of ambient illumination that enables the animal to see the relevant visual stimuli or in the form of visual displays that are comprised of lights. The light requirements for visual tasks can vary enormously depending on the species and the task that are studied. For example, rhesus monkeys are very similar to man in most of their visual functions including acuity (Covey & Ellis, 1967), stereoacuity (Sarmiento, 1975), luminosity and color vision (DeValois et al., 1974a), and visual spatial abilities (DeValois et al., 1974b, Smith et al., 1982), while the rat's visual function is significantly different. Unlike humans and monkeys, rats have virtually no color vision (Muntz, 1967). Consequently, a color discrimination task that would be simple for a monkey is nearly impossible for a rat.

In attempting to design lighting systems for future space missions, we cannot anticipate the exact task-species combinations that will be studied, we can identify two lighting parameters that might act as serious constraints for visual tasks. They are the intensity and spectrum of the ambient illumination. Most important, visual acuity is significantly reduced at low intensities, so that very low intensity lighting could seriously limit the stimuli that could be used in visual tasks. Also, the use of monochrome or narrow spectrum light sources could seriously performance on tasks where color is an important dimension. However, neither of these considerations should present serious problems given the design currently under consideration.

In addition to its direct stimulus effects, light can also exert powerful contextual effects on learning and performance. Contextual effects in learned tasks arise because, in addition to the stimuli and contingencies involved in the task per se, the animal learns about the static stimuli surrounding the task. In addition to what happens, it learns about where it happens. The most common type of contextual effect involving light occurs when the light condition during testing is different from the light condition

during training. Evans and Patton (1970) found that training and testing in different light conditions could produce greater impairment than scopolamine, a drug that is frequently used to induce memory loss.

Context effects of lighting can also participate in more complicated effects on learning and performance. Tomie (1976) showed that the light conditions could produce "blocking" that significantly retarded autoshaping of a bar pressing response. Blocking is a complex, attention-like phenomenon, where the previous experience conditions the animal to "ignore" some of the relevant stimuli in the current task (Kamin, 1969). In the terms of typical blocking explanations, the animal learns that the ambient lighting condition in the apparatus effectively predicts the occurrence of the unconditioned stimulus (food presentation), so that it does not learn to respond to subsequent additional cues provided by the autoshaping stimuli.

Most of the contextual effects of light can be avoided in one of two ways. First, animals should be trained and tested in the same lighting environment that will be used in flight. This precaution will prevent impaired performance due to context generalization decrement. Second, context effects like those in the blocking experiment can be prevented by explicitly extinguishing the animal to the task context. This is done by repeatedly presenting the task light conditions in the absence of unconditioned stimuli. In addition, contextual effects are a much greater potential problem if the lighting system is very distinctive. Therefore, contextual effects can be reduced or avoided if we avoid the use of very distinctive lighting such as narrow spectrum light.

Problems with the direct stimulus effects of light and problems with the light context can be avoided through some common sense considerations in the design of experimental modules and through some thoughtful precautions in procedures used on the ground and in flight. However, light can also influence performance through indirect effects on a variety of biological and behavioral systems.

An important group of effects appears to be the result of a non-specific arousal effect of light. A good illustration of these effects is seen in an experiment by Hornbuckle (1972). Hornbuckle found that diurnal squirrel monkeys responded significantly faster on a delayed response task when it was tested in the light than when it was tested in the dark. In contrast, she found that nocturnal owl monkeys performed significantly faster in the dark than in the light. Thus, each species performed better in the light conditions under which it is normally active. Similar results have been seen in other tasks and measures such as fixed interval responding and vigilance (Goethe & Isaac, 1977; Isaac, 1969; Delay & Isaac, 1980; Isaac and Kallman, 1979). These light effects even appear to extend across sensory modalities with squirrel monkeys showing significantly lower auditory thresholds in the light than in the dark (Delay, Steiner & Isaac, 1979).

Isaac and his colleagues have suggested that these effects are due to non-specific activating or arousing effects of light. Thus, in the delayed response experiment above, Hornbuckle (1972) found that a non-light activating stimulus (white noise) produced increased response speed in both diurnal squirrel monkeys and in nocturnal owl monkeys. Another piece of evidence for this arousal hypothesis is that stimulants, methyphenidate and d-amphetamine interact with the light effect (Delay & Isaac, 1980; Delay, Steiner & Isaac, 1979).

Another avenue for indirect light effects on performance is through the circadian system. In addition to simple circadian performance rhythms, Holloway and his colleagues have reported fluctuations in retention deficits

that are synchronized to the time of original training. They found that retention was good 15 min after training and at 24 hr intervals after training. Retention was also moderate to good 12 hrs after training and at 24 hr intervals thereafter (12 hr, 36 hr, etc), but retention was poor at 6 hrs and 18 hrs after training and at 24 hr intervals after those times (6 hrs, 18 hrs, 30 hrs, 42 hrs, etc) (Holloway & Wansley, 1973a,b). However, these fluctuations in retention could not have been due to simple circadian performance or retention rhythms because similar curves were found regardless of the time of day when training occurred (3 a.m., 9 a.m., 3 p.m., or 9 p.m.). Similar results were found for a variety of appetitive and aversive tasks (see Holloway, 1978 for review).

To explain these results, Holloway suggested that the animal is able to use its circadian system to provide an internal context cue that depends upon an endogenous physiological state at the time of training. That internal state is repeated most closely at 24 hr intervals. So the animal's performance is best at 24 hr intervals following training when the internal context associated with training is repeated. In contrast, the animal's retention is poor at 6 hrs, 18 hrs, 30 hrs, etc when the animal's internal physiological context is different from the internal state that existed at the time of training. The 12 hr training-testing intervals represent an intermediate case. Thus, the circadian system can provide an internal retrieval cue that enables the animal to access learned information.

Circadian effects are not restricted to rhythmic fluctuations in retention. Manipulating the circadian system by phase shifting the light-dark cycle can produce retrograde amnesia (Tapp and Holloway, 1981). In that study, we found that 6 or 12 hr phase shifts of the light-dark cycle imposed immediately post-training produced retrograde amnesia in rats trained on a passive avoidance task. The memory deficit persists after the animal has completed reentraining to the new light-dark cycle so that the deficits are not due to transient performance deficits associated with experimental jet-lag. Further, the deficits were not due to changes in illumination at the time of testing because deficits occurred when animals were tested following a 6 hr phase shift that was arranged so that both training and testing fell in the light phase of the cycle. This finding is striking because most things that produce retrograde amnesia are very traumatic such as blows to the head, electroconvulsive shock and some drugs. The fact that a simple, well-timed, light-dark change can produce similar effects suggests that these circadian manipulations are affecting some important aspect of the system.

This and other considerations led Ben Natelson and I to develop a system for studying circadian effects on performance in rhesus monkeys. In this system, rhesus monkeys are instrumented so that we can collect body temperature and activity data around the clock. This arrangement enables us to monitor their circadian rhythms as we impose different experimental manipulations on them. The monkeys are trained to perform a chained vigilance-discrimination task to obtain their food. We chose vigilance and discrimination because many real life tasks involve some compound requirement for sustained attention and performance (vigilance) and a requirement for a choice or selective action based on information at-hand (discrimination). Two major themes have emerged from this work: (1) that manipulations of light and circadian rhythms can have important effects on performance and (2) that these performance effects depend upon the type of task.

The vigilance-discrimination apparatus consists of a cue light with three levers beneath it. A vigilance trial begins when the cue light is illuminated white. The monkey then has 10 sec to press the center lever. If

he completes the vigilance trial successfully, the cue light changes to red or green as the discrimination trial begins. If the monkey chooses the correct lever (right or left) in the discrimination trial, he gets reinforced with a banana-flavored Noyes pellet. The monkeys obtain all of their food in this task so their performance becomes quite good.

In the first set of studies, monkeys worked on the task for 8 hrs/day beginning one hour after lights on. Figure 1 shows average performance patterns across the 8 hour session. Performance is expressed as reciprocal response latency * 1000 (a measure of response speed) so that performance improves as the measure goes up. The solid line shows vigilance performance and the dashed line shows discrimination performance.

Discrimination performance is best at the beginning of the session and declines over the course of the day, while vigilance performance begins at moderate levels, improves to an afternoon peak and then declines in the late afternoon. The difference in peak times for the two rhythms is significant ($p < 0.01$). The task-dependent time of day patterns on these two tasks were similar to time of day performance patterns reported in humans. In the human data, simple repetitive tasks requiring low short-term memory (STM) loads peaked in the afternoon near the time of the temperature peak while more complex cognitive tasks peaked early in the day and exhibited a negative correlation with body temperature (Folkard et al., 1976). In the monkeys then, vigilance appeared to behave like a simple low- to intermediate-memory task, while discrimination appeared more like the cognitively complex high memory tasks.

Task-dependent differences were also found in the effects of experimental jet lag that was produced by phase-shifting the monkey's light cycle (Tapp & Natelson, submitted). As in human jet lag, phase shifting the light cycle produced significant performance decrements in monkey vigilance ($p < 0.001$) and discrimination ($p < 0.001$) for several days following the shift. Figure 2 compares the average deficit in vigilance and discrimination following a 6 hr phase advance. The plot shows that the maximal vigilance decrement produced by jet-lag was significantly more severe (49.1%) than the maximal discrimination decrement (11.3%; $p < 0.01$). Similarly, the vigilance decrement lasted significantly longer than the discrimination deficit, with vigilance significantly impaired for an average of 5 days \pm 0.57 SEM following the phase shift and with discrimination impaired for an average of 2.33 days \pm 0.57 SEM days ($p < 0.01$). These results are similar to task dependent differences reported for humans during jet-lag (Klein, et al., 1972).

In addition to the expected deficits that immediately followed the phase shift, we found a second, later deficit that was not anticipated based on any previous data. This second deficit occurred 12-14 days following the phase shift was smaller than the initial jet lag effect, but it was significant for both vigilance ($p < 0.01$) and discrimination ($p < 0.01$). This deficit is remarkable, in part, because it occurs several days after performance returns to normal levels. Prior jet lag studies have not examined daily performance on a detailed basis this long after the phase shift so it is not surprising that this second period of impairment has not been reported previously. If similar, second deficits occur in humans at this time -- a time when performance is currently expected to have returned to normal, it would have important operational consequences. We believe that this possibility should be carefully examined.

The results described above demonstrated that monkey performance on the vigilance-discrimination task modelled many of the important features of

human performance in circadian experiments. As a next step we investigated performance on the 8 hr task in constant light.

To our surprise, the 8 hr vigilance-discrimination task acted as an effective entraining agent that synchronized the circadian activity and temperature rhythms as well as the patterns of performance (Tapp et al., 1986). Thus, in constant light, the temporal patterns of performance as well as the circadian temperature and activity rhythms were essentially the same as in the 24 hr entrained animal. Despite these similarities in pattern, monkeys living in constant light exhibited significant overall performance decrements in both vigilance and discrimination (Tapp et al., 1985) which are depicted in Figure 3. The constant light deficits were unlike the deficits associated with jet lag in two important ways. Unlike the transient jet lag deficits, the deficits in constant light were long lasting. They lasted as long as the animals were maintained in these conditions. Second, the impairment was about the same magnitude for both vigilance and discrimination in constant light, while there was a marked difference in the magnitude of impairment following jet lag, with vigilance more impaired than discrimination. At present, we cannot be certain what aspects of constant light produce these performance deficits, however, they are further evidence of the fact that the lighting environment can have an important impact on performance.

In summary, the lighting environment can have a significant impact on learning, memory, and performance. While some aspects of the lighting requirements may be so task- and species-dependent that they would only be specified as part of certain experimental protocols, the results presented here suggest that the following general considerations in the design of environmental lighting in the habitats would make it easier to implement learning, memory, and performance studies without additional modification. First, contextual effects of light can have marked effects on learning and performance, but most contextual effects can be avoided by training the animal in the same lighting context that it will experience in flight or by explicitly extinguishing the animal to the lighting context. Second, light appears to have indirect effects on learning, memory, and performance mediated through light effects on arousal and through light effects on circadian rhythms. In order, to avoid the potential pitfalls that might be produced by these effects, it seems desirable to provide the capacity for stable 24 hr light-dark cycles. This provision would enable the experimenter to have confidence that training and testing conditions can be standardized on a day-to-day basis and would avoid the potential problems associated with phase shifts which can be shown to produce performance deficits and retrograde amnesia. With the availability of a stable, ambient lighting cycle, many learning, memory and performance experiments might be implemented in the microgravity habitats with little or no special lighting modifications necessary.

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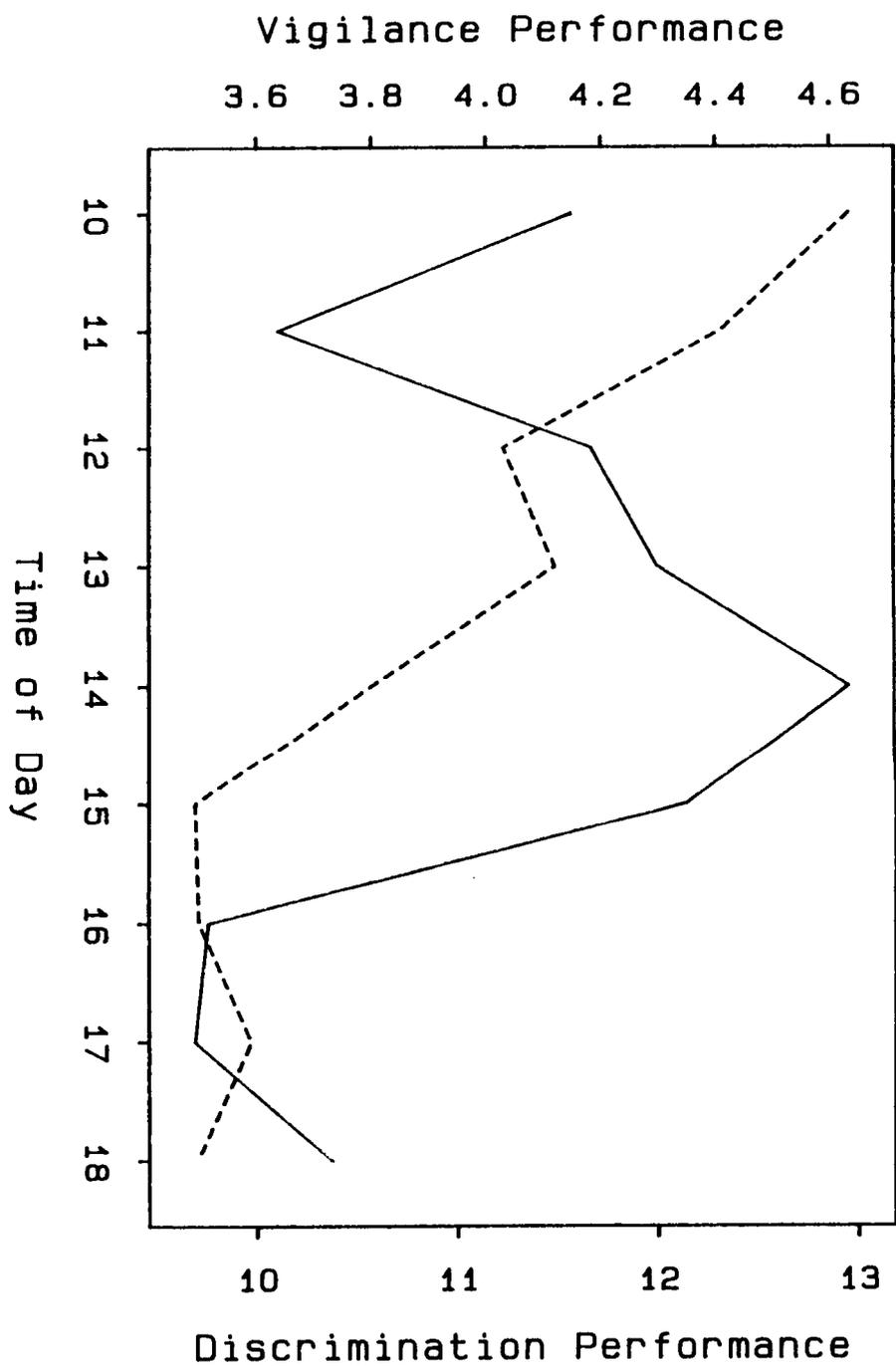


Fig. 1 Average performance patterns versus time of day.

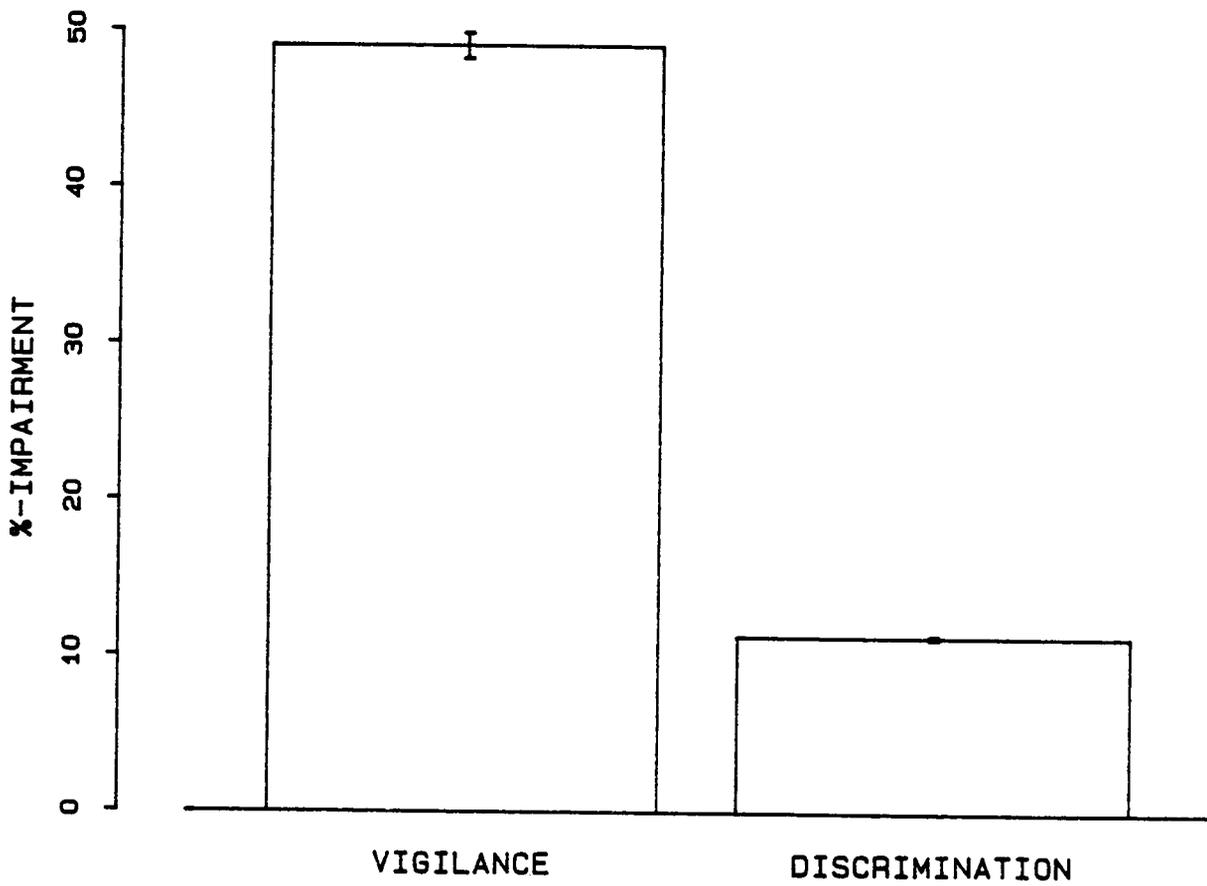


Fig. 2 Vigilance and discrimination performance following 6 hr phase advance.

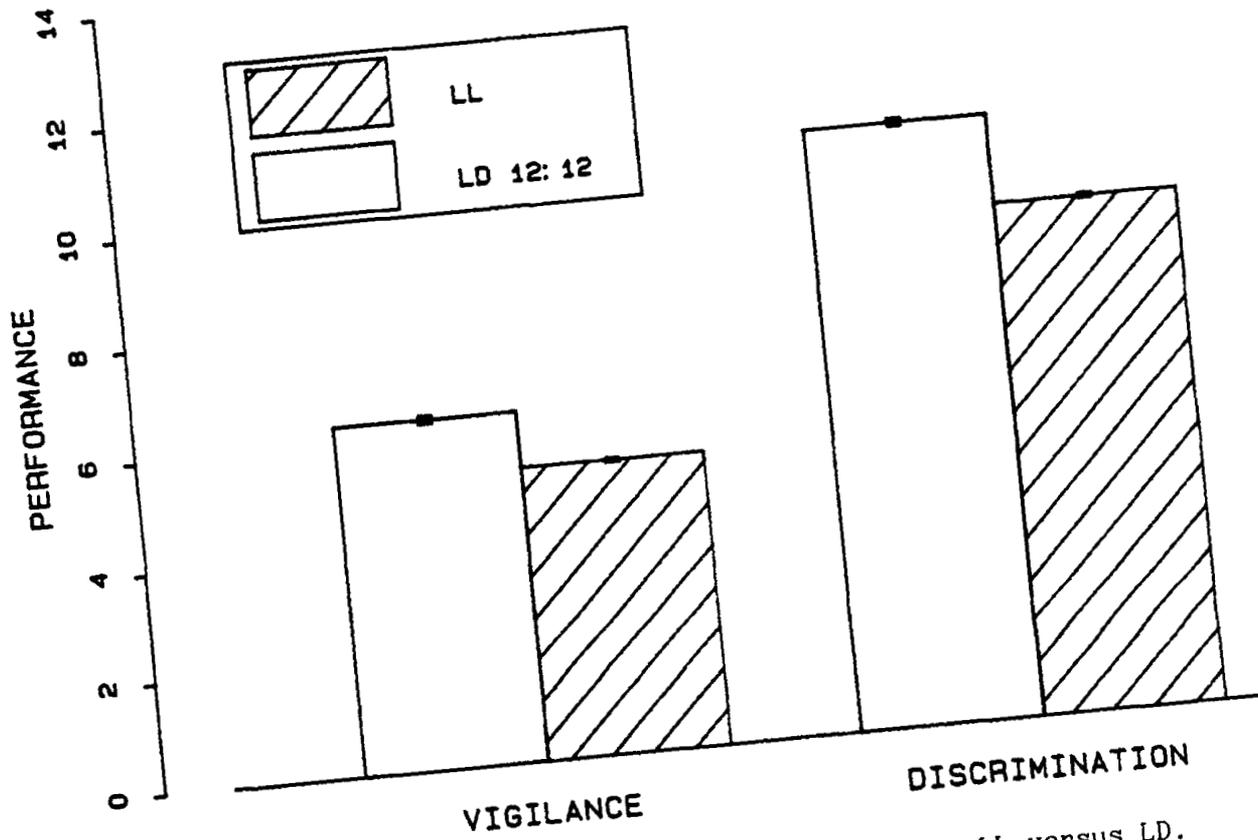


Fig. 3 Vigilance and discrimination performance in LL versus LD.

Comparative Aspects of the Effects of Environmental Lighting†

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Key Words: rhythms, circadian, reproduction, photosensitivity, phase-response curve, wavelength, illuminance, entrainment, non-parametric model of entrainment, feedback lighting, fungus, rodent, mice, rat, hamster, primate, squirrel monkey, rhesus monkey

Comparative Aspects of the Effects of Environmental Lighting[†]. James S. Ferraro, Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, Illinois 62901.

Abstract. Many behavioral and physiological functions characteristically operate with relatively stable diurnal periodicity. These rhythms time locomotion, performance, sleep-wake and temperature cycles, hormonal secretion, ovulation, sexual receptivity, breeding season and numerous other processes. Several characteristic features of these circadian (*Latin*, about a day) rhythms distinguish them from other types of rhythmic phenomena in biology. When an organism is removed from obvious environmental cues and placed in temporal isolation (i.e., in constant temperature, illumination or darkness) these rhythms can persist indefinitely with a period of approximately, but not exactly, 24 hours. The endogenous pacemaker (or clock) is entrained by the periodicity of the environment, most importantly the light-dark cycle imposed by the earth's rotation. A remarkable feature of these circadian rhythms is that the endogenous period is remarkably constant irrespective of the environmental temperature (i.e., these oscillations have a Q_{10} very close to 1). With the exception of prokaryotes, these rhythmic phenomena are found in all major classes of plants and animals (53).

In the wild, organisms are exposed to the environmental cues of light and dark and thus can adjust their biological clock to precisely entrain it to the solar day. This is made possible by the existence of variability in the photo-responsiveness of the circadian oscillators to light signals falling throughout the circadian cycle, as defined by the phase-response curve. This phase-response curve is surprisingly similar across species. Furthermore, not only is the phase of light important in biological responses, but the quality of light is crucial; light of different intensities and/or wavelengths can not only produce different, but opposite effects. A self-generating and environmentally correctable, timekeeping mechanism is crucial to the organisms very survival. If an animal is to: look for food at a time when it is available and when competition is reduced; search for a mate at a time the mate will be aroused and available; and to perform these functions when the risk of predation is diminished, than a sense of time is imperative. These diurnal rhythms have also been shown to be critical to human performance and function, affecting systems as diverse as drug tolerance/effectiveness to reaction time. Light, and its effect on the endogenous timing mechanism, is as important to all parameters of experimental plant and animal studies, as it is to the experimenter/astronaut himself.

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Introduction

The environment in which we live undergoes a reliable and predictable rhythm in many geophysical variables; the most readily apparent of these rhythms are the cycles of light and dark, and higher and lower temperatures. These geophysical rhythms have important influences on food availability, metabolic costs, predator activity and mate availability. It would be of grave importance, therefore, that an organism be able to know when these changes in the environment were to occur. It would be even more beneficial if the organism could anticipate or predict these variations. In order to predict these changes, an organism would have to necessarily possess a sense of time. In the best of cases, its oscillation should approximate the environmental 24 hour period; furthermore, the oscillator should be able to tell time independently of the environmental variables, and yet use these variables to correct the phase of its own oscillation. An added bonus would involve being able to anticipate seasonal changes in the environment, in addition to the daily variations.

To qualify as a self-sustained endogenous circadian pacemaker, an organism's oscillation(s) would have to be capable of persisting even when the organism is exposed to constant conditions (light, temperature, etc.), isolated from the environmental time cues (Zeitgebers). The first experiment in which an oscillation was measured in the absence of Zeitgebers was performed on the petal movement of the sensitive heliotrope *Mimosa pudica* (15). The daily rhythm of leaf opening and closing persisted indoors, inside light tight boxes devoid of environmental cues. More than 100 years later, de Candolle repeated the experiment and discovered that the leaf movement free-ran with a period slightly shorter than twenty-four hours (13). This result further strengthened the hypothesis that this rhythm was endogenously driven (53).

Since these studies, endogenously generated self-sustained circadian rhythms have been found to exist in most eukaryotic organisms. Even single cell organisms, such as *Gonyaulax polyedra*, possess endogenous rhythms (42). These persisting rhythms control many physiological and behavioral functions and are found in both plant and animal kingdoms, from the flower opening of the water lily to the daily color change in the fiddler crab. Circadian rhythms also control the timing of rhythms in humans, such as daily cycles of sleep-wake, activity, temperature and sodium excretion. Disturbances in the phase relationship between these endogenous oscillations and the external environment occur in situations of transmeridian jet travel and shift work schedules (53). Such disruptions in phase relationships can lead to decreased performance, sleep disturbances etc.; alterations in the circadian oscillator's phase relationship has even been implicated in certain forms of depression in humans (81).

A second important characteristic of an endogenously generated self-sustained timing system is the ability to correct its endogenous timekeeping so that it maintains the correct phase relationship with the environment. This is accomplished by a differentially photosensitive phase

shifting mechanism. This variation in the oscillators sensitivity to light, and the direction in which the system responds, is the heart of the entrainment process, where the period of the organism's pacemaker (τ) is made equal the period of the environmental oscillation (T). This differential photosensitivity can be experimentally measured and graphically represented as a phase-response curve (PRC). The effect of light on circadian oscillations is not restricted to the phase of the light, but is also dependent on other characteristics of light, such as wavelength, intensity and in certain cases, pulse duration.

Plants and animals also possess oscillations which are about a year in length. These circannual rhythms, such as the blooming of long (e.g., black-eyed Susan) and short (e.g., violet) day plants and breeding seasons of long (e.g., Syrian hamster) and short (e.g., ram) day mammals, are also entrained to the environmental time cues of light and dark (e.g., photoperiod length) and are dependent on circadian oscillations (18, 20, 23, 41, 56, 57, 71).

Methods and Materials

Fungus

Neurospora crassa

The filamentous fungus, *Neurospora crassa*, displays rhythmic growth patterns in certain specific conditions. This rhythmic growth consists of an alternating low growing surface mycelium and a surface mycelium with aerial hyphae which pinch off to form conidia (asexual spore formations). The mycelium that contains the aerial hyphae is clearly seen as a band when grown on media in petri dishes or long cylindrical tubes, known as "race" tubes. In constant conditions the cycle of banding is repeated approximately once every 21.5-22 hours (i.e., the rhythm is in the circadian range) (24).

Neurospora crassa band strain (#1859) and band CSP strain (#4548) were used. Stock cultures are grown on slant tubes containing Vogel's salts, 1.2% sodium acetate and 0.5% Difco casamino acids. Experimental cultures are grown by inoculating one end of a "race" tube containing medium. Cultures are grown in constant bright light for 24 to 48 hours, then the growth front is marked on the glass tube and the tubes are placed in constant darkness. In the laboratory, the growth fronts are marked at regular intervals during the course of an experiment. Marking is done under a red "safe" light which has been shown not to affect the free-running conidiation rhythm. At the end of the experiment the growth front is again marked. Since growth is linear at constant ambient temperatures, the time of occurrence of each conidiation band can be determined from the growth front marks. These marks are recorded by a digitizer and analyzed on an Apple IIe microcomputer.

Ground based experiments utilized: 1) a clinostat (rotating the tubes in a 1G vector in order to evenly distribute the force and obtain a simulation of zero G), 2) different orientations of the 1G gravity vector, 3) chronic and acute exposure to hypergravity (3G), 4) genetic strain, and 5) media composition on the rhythmic conidiation and growth of *Neurospora*. To determine if this

circadian rhythm of conidiation is endogenously derived or is driven by some geophysical time cue, an experiment was conducted on STS-9 where race tubes inoculated with growing *Neurospora* were exposed to the microgravity environment of space. The results demonstrated that the rhythm can persist in space; however, several aberrations were noted (74, 75).

Rodents

General

Syrian hamsters (*Mesocricetus auratus*), rats (*Rattus norvegicus*) and mice (*Mus musculus*) were purchased from Charles River Breeding Labs (Wilmington, MA). Syrian hamsters were maintained in a light-dark cycle of 14 hours of light and 10 hours of dark (LD14:10) prior to experimental exposures, while rats and mice were maintained in LD12:12. Djungarian hamsters (*Phodopus sungorus*) which were derived from a stock generously supplied by Dr. Bruce Goldman at the Worcester Foundation, were maintained in LD16:8 prior to experiments. Food and water were provided *ad libitum* throughout all the experiments.

Syrian hamsters

Male Syrian hamsters were placed in individual light-tight, sound attenuated chambers and exposed to one of five lighting conditions for a duration of 10 weeks: constant light (LL), constant dark (DD), feedback lighting (LD_{FB}), a feedback lighting neighbor control (LD_{FB} NC) and reverse feedback lighting (rLD_{FB}). These lighting conditions are described below. A second group of hamsters were also exposed to one of the following lighting conditions for 10 weeks: LD14:10 (a long photoperiod); LD10:14 (a short photoperiod); an ultra-high frequency or ultrashort light-dark cycle of 1 minute of light followed by 1 minute of dark (LD1m:1m); and a high frequency light-dark cycle of 1 hour of light and 1 hour of dark (LD1:1). The experimental chambers were illuminated by a 15-watt incandescent bulb providing an illumination of 45-75 lux. Each chamber contained a Wahmann activity cage, with a 13.5 inch diameter running wheel and an adjoining cage where the animal had free access to food and water. The illumination in the DD cages was below detectable levels (0 lux). Maintenance of the animals was performed in ambient (available cage lighting) or dim red (<610nm) light on a daily basis. The time of day that the maintenance was performed was varied and did not affect the free-running rhythms of the animals.

Feedback lighting (LD_{FB}) is a condition in which an animal receives light in response to a given threshold of locomotor activity (34). In these experiments, the light was turned on for 1-2 minutes when a wheel running rate of nine revolutions per minute was obtained. If nine revolutions of the wheel were not performed within 1-2 minutes of the first revolution, the counters and timers returned to zero without power application to the outlet. Consequently, most of the light exposure occurred during the subjective night and was highly correlated with activity.

The hamsters in neighbor control lighting ($LD_{FB} NC$) had no control over their own lighting condition; but instead had their lighting condition determined by a randomly paired animal which was exposed to LD_{FB} . Thus, the $LD_{FB} NC$ animal was exposed to the identical lighting profile as its LD_{FB} controller. Unlike the controller, however, the $LD_{FB} NC$ animal could assume a different phase relationship with the lighting schedule. The light exposure of this lighting condition illuminated portions of the subjective night relatively frequently; however, considerably less than the controlling hamsters (LD_{FB}) (25, 26).

Reverse feedback lighting (rLD_{FB}) is identical to LD_{FB} in all aspects save one: the cage lights are on continuously unless the animal runs. If the animal runs with a revolution rate of at least 9 wheel turns within 1-2 minutes of the first revolution, the light in the animal's cage turns off. If the ninth revolution is not completed in the allotted time, the timers and counters reset without interrupting power to the light (27, 30).

Constant light (LL) was chosen as a photoperiodic simulator of long days (LD14:10), since it can produce maintenance and recrudescence, while allowing the circadian oscillator to free-run (the circadian oscillator also free-runs when the animal is exposed to LD_{FB}). Constant dark (DD) was chosen as a simulation of short photoperiods (LD10:14), since it allows a free-running circadian oscillator accompanied by testicular regression. Short and ultra-short LD cycles (LD1:1 and LD1m:1m) were used to determine the effects of multiple LD transitions.

Locomotor activity was continuously monitored and recorded in half hour bins by an Apple II microcomputer as previously described (73). Raw data were converted into locomotor activity records (actographs) by placing each day's record below the previous day's record, then duplicating the record and placing them side by side so that a 48-hour consecutive record is represented on the X axis and consecutive days on the Y axis (see Figures 2 and 3). The period of the activity rhythm was calculated by the computer by performing a linear regression on the acrophases of computer generated sine waves that had been fitted to the data. This method has been shown to be in good agreement with eye-fitted hand drawn lines through the onsets of activity on the plotted actographs (35).

At the end of the experimental exposure, the animals were lightly anesthetized with ether, weighed and decapitated. Trunk blood was collected immediately and refrigerated. Whole blood was centrifuged on the day of autopsy; the serum was collected and frozen until the day of hormonal assay. Serum testosterone (T), prolactin (PRL), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by radioimmunoassays (RIA). Testes, accessory reproductive glands (seminal vesicles and coagulating glands) and adrenal glands were removed and weighed after blotting. If the seminal vesicles contained fluid it was noted and removed before weighing. Sections of the testes were obtained to determine the phases of spermiogenesis and diameter of the seminiferous tubules. An arbitrarily chosen testis from each animal was

fixed, sectioned and stained. The most advanced phases of spermiogenesis (9, 51) were determined for each animal. The presence or absence of degenerative germ cells was also noted. Five arbitrarily chosen seminiferous tubules in the upper left quadrant of each slide had their diameter measured.

Female Syrian hamsters were examined daily for the "positive sign of ovulation" (i.e., a stringy massive vaginal discharge of nucleated epithelial cells on the day of estrus; 12). Vaginal washings were also taken and examined for pH, appearance of calcium carbonate concretions and cytology (1, 2, 31). At autopsy mean uterine and adrenal glands weight were determined, the ovaries were examined for follicle and corpora lutea presence and size and the oviducts were flushed in an effort to recover ova.

Immature Djungarian hamsters

Immature male Djungarian hamsters were weaned and sexed on the 18th day of life. Males were weighed and placed inside individual light-tight sound attenuating chambers. Each chamber, ventilated by a fan, could be illuminated by a 15 watt soft-white incandescent bulb which provided approximately 200 lux of illumination. The chamber contained a cage equipped with food, water, wood shavings, and an activity wheel. The animals were divided into four lighting groups; DD, LL, LD_{FB} and LD_{FB} NC. A fifth group of twelve additional males were group housed (2-4 per cage) in the colony room (LD16:8, 22 lux) and did not have access to running wheels. LD16:8 was used to verify that indeed LL simulates the reproductive effects of long days.

Animals were removed from the lighting conditions after being exposed for approximately 30 days (i.e., the 48th day of life). The animals were immediately sacrificed at which time the pelage color (37, 47) and total body weight were assessed and recorded. Pelage color was divided into shades ranging from stage 1 (a primarily black and gray, summer color) to stage 6 (a mostly white, winter color). Testes, combined sex accessories (seminal vesicles and coagulating glands) and adrenal glands were removed and weighed wet after blotting. Tissue weights were corrected for differences in body weight and are presented in mg/10g total body weight. This correction was utilized to accurately compare tissue weights despite concomitant photoperiodic changes in total body weight (47). It was also determined if the seminal vesicles contained fluid (if fluid was present, it was removed before the tissue was weighed).

Mature Djungarian hamsters

Mature male Djungarian hamsters were raised in a colony room with a light-dark cycle of LD16:8. A group of hamsters were exposed to LL, DD, LD_{FB} and LD_{FB} NC for 41 days and a second group exposed for 71 days. The hamsters exposed to these lighting conditions were individually housed in ventilated, light-tight, sound-attenuated chambers with an interior light intensity of 200 lux. Each animal had access to an activity wheel. All hamsters exposed to LD16:8 were

exposed for 40 days, did not have access to activity wheels and were group housed in the colony room with a light intensity of 22 lux. A tenth group of hamsters were group housed in a third room and were exposed to LD1:1. These animals also did not have access to activity wheels. The intensity inside this room was 240-410 lux.

Following the lighting exposure, animals were sacrificed and weighed. Pelage color was determined as previously described (37) and testicular, sex accessory glands (seminal vesicles and coagulating gland), and adrenal gland tissue was removed, blotted and weighed. The amount of fluid in the seminal vesicles was noted and removed before weighing. Due to the photoperiodic effects on total body weight (TBW) the reproductive and adrenal tissue measurements are presented in mg/10g TBW (47).

Rats and Mice

Female rats and mice were treated similarly to the male and female hamsters. Each animal was housed in an individual cage with a wheel and was exposed to LD12:12, DD and various intensities of LL and LD_{FB}. Locomotor activity was monitored and period, total activity and alpha were determined.

Phase-response and Aschoff illuminance curves were developed for the rat and compared with curves from other organisms. To develop a phase-response curve, rats were exposed to continuous darkness for two weeks and then given a 60-minute light pulse with an illuminance of 150 lux. The animal was allowed to free-run in DD for an additional two weeks. Pulses were given at two week intervals throughout the circadian cycle. Advances and delays were estimated to the nearest quarter hour by measuring the deviation of the onset of running after the pulse from that predicted from the length of the period immediately preceding the pulse. Transients were allowed to be completed before the estimate was made. Aschoff illuminance curves were determined by measuring the period length of the locomotor activity rhythm under different intensities of constant light.

Vaginal cytology was also monitored daily in order to determine whether the animals were cycling reproductively. At autopsy, uterine and adrenal glands weight were determined. Ovaries were examined to determine the presence and size of follicles and corpora lutea.

Primates

Saimiri sciureus

Adult male squirrel monkeys (*Saimiri sciureus boliviensis*) were individually housed in cages which were placed in light-tight sound attenuating chambers. Food and water for these diurnal primates was available *ad libitum* throughout the experiment and were replenished daily at varying circadian times. Each chamber was individually ventilated through light traps. Light (600 lux), whether for LL or LD_{FB}, was provided by a 12 inch 32 watt round fluorescent fixture.

When the animal was placed into continuous darkness (DD) the intensity inside the chamber was below detectable levels (0 lux). Seven of the eight exposures of animals to LD_{FB} were followed by constant conditions (i.e., LL or DD) in order to determine if the free-running drinking rhythm was a true reflection of the endogenous oscillator and not a result of masking due to the previous lighting conditions.

Drinking rhythms were recorded continuously in 0.5 hour bins on an Apple II microcomputer (73). Drinking was monitored through the closing of an electric circuit when the animal came in contact with the drinking spout. The drinking counts were converted to milliliters of water consumed in mls per 0.5 hour.

Some animals were monitored for activity and core body temperature as well as for drinking. Activity and temperature data were also obtained in 0.5 hour bins through the use of an intraperitoneally implanted AM transmitter; as described elsewhere (43). The frequency output paralleled temperature and the amplitude output defined activity. The frequency telemetry data was converted to temperature degrees C. The temperatures have been estimated to be accurate to ± 0.2 °C.

Feedback lighting (34) was controlled by electronic circuits which were modified in order to illuminate the SN of this diurnal monkey. In previous experiments with nocturnal rodents, the monitored behavior (wheel running) turned the environmental light on to illuminate the SN. In this experiment, the monitored behavior (drinking) turned the environmental cage light off whenever the animal drank for several seconds. The lights remained off for approximately 10 minutes following the completion of the drinking bout. This modification of the LD_{FB} circuit resulted in the major light exposure during the SN.

Computerized reductions of raw data were performed. The data points, corresponding to a given half hour bin of the data for each day of the lighting condition, were averaged. The mean \pm SEM for each half hour bin was then plotted, resulting in the average waveform of the oscillation for a given condition. The results of such an reduction allows one to more easily compare wave forms across conditions.

Macaca mulatta

In all of the spaceflight (COSMOS 1514) and synchronous and vivarium control ground based studies, young (3 to 5 Kg) rhesus monkeys (*Macaca mulatta*) were used (76). Before collecting data the monkeys were extensively trained for periods of 2-6 months to accept restraint in metabolism chairs for periods of 1 to 2 weeks. Activity was monitored via a sensor attached to the monkey's restraint jacket. Axillary temperature was monitored with a Soviet biotelemetry system. Ankle skin temperature was measured by YSI thermistors. Ground baseline data were collected at 1G with the same procedures used in the spaceflight experiment. However, in some of the experiments conducted in the U.S. ankle temperature was measured with copper-constantan

thermocouples. Axillary temperature was also monitored with surface thermocouples taped to the axilla, and these data were identical to the axillary temperatures measured by telemetry. Colonic temperature was also monitored with thermocouples to evaluate how well axillary temperature reflected core body temperature. Oxygen consumption was continuously monitored with an oxygen analyzer. The monkeys were placed in a plexiglass chamber that was approximately 80 cm X 20 cm, and 8 liters/min of air were pulled through the chamber. The air flow maintained the chamber oxygen above 20%, carbon dioxide below 1%, and the relative humidity between 50 and 60%. The outflow air was dried, carbon dioxide removed, and then passed into the oxygen analyzer. Heart rate was measured by a Vitalog PMS-8 solid state recorder.

Statistics

Statistical comparisons were made using a one-way analysis of variance. Pairwise comparisons were then made using the Duncan multiple range test. Linear regressions were performed and correlation coefficients calculated.

Results

Most eukaryotic organisms demonstrate fluctuations in behavioral and/or physiological functions; furthermore, most of these organism's rhythms can persist even in the absence of temporal cues from the environment (3, 69). Phase-response curves (PRC) depict experimentally derived photosensitivity relationships and suggest mechanisms of entrainment in the given organism (10, 14, 54, 55). PRCs have been determined for a wide variety of species and the general shape of the curves are quite similar (10, 14, 44, 52, 54, 55, 67, 72). Figure 1 shows PRCs for the albino rat, the Syrian and Djungarian hamsters and the squirrel monkey. Despite the obvious differences between rodents, and primates and between nocturnal and diurnal species, the PRCs are remarkably similar. The photosensitivity of these animals is characterized by phase delays in response to light in the early subjective night, phase advances in the late subjective night and relative insensitivity during the subjective day when the major light exposure would occur in nature. Even the PRC of *Drosophila*, *Neurospora* and *Gonyaulax* are qualitatively similar to the mammalian curves. These diverse organisms utilize this variability in photosensitivity in order to entrain the period of their endogenous oscillators with the 24-hour period of the environmental Zeitgebers, such as light. Entrainment mechanisms have been shown to be amazingly similar in extremely diverse species (10, 14, 42, 44, 52, 54, 55, 67, 72).

Since, these PRCs suggest that organisms are relatively insensitive to diurnal light exposure, it has been hypothesized that light exposure during the subjective night should be capable of producing most, or all, of the effects observed in constant light (25). Feedback lighting (LD_{FB}) is a lighting condition in which the organisms own behavior triggers the environmental lighting. The system can be altered so that this endogenously triggered light exposure will illuminate the

subjective night regardless of whether the animal is nocturnal or diurnal in its activity preference.

Figure 2 shows double-plotted actographs which depict female rats exposed to LL or LD_{FB}; in both lighting conditions the free-running period is lengthened to a similar extent (33). The fact that animals exposed to LL and LD_{FB} have similar free-running periods has been shown in several nocturnal rodent species and one diurnal primate (see Figure 3 and Table 1; 25-27, 30-33, 35, 36). Similar effects have been observed in more ecological laboratory environments (58). To determine whether the effects of LD_{FB} were affecting the rhythms by masking, rather than truly free-running, animals were released into constant conditions. All animals free-ran from the predicted phase relationship suggesting that LD_{FB} had not masked the rhythm. In the experiments utilizing diurnal primates, three rhythms were monitored simultaneously in an effort to determine whether LD_{FB} abnormally altered the phase-relationships between different rhythms. The results shown in Figure 4 demonstrate that the phase relationships in LL and LD_{FB} were stable and similar (35). This relationship between the period lengthening effects of LL and LD_{FB} was not absolute. As the pulse duration of LD_{FB} was decreased, the effects diminished, suggesting a temporal limit to the effects of pulsatile light and therefore, the non-parametric model of entrainment (Figure 5; 32). This limit, however, was at very short pulse durations and may not have an important ecological aspect.

The similarity of period lengthening effect of LL and LD_{FB} also extends to different intensities. Most nocturnal and some diurnal organisms have increased free-running periods in increased intensities of LL. This well known Aschoff intensity effect of LL is found in many different species (Figure 6; 10, 44, 52, 72). This effect can also be simulated by exposure to LD_{FB} (see Figure 7; 32, 33).

We also examined the effects of LD_{FB} on reproduction in the albino rat. The reproductive cycle of rats is known to depend on circadian oscillations and that in bright constant light, the female rat will stop showing 4-5 day estrus cycles (33). In these conditions, the rat, will change from a cyclic ovulator into a reflex ovulator (i.e., the rat will ovulate in response to copulation as opposed to spontaneously ovulating every four or five days). This condition of persistent estrus increases in frequency with an increase in intensity. Again, it was felt that if the organism's circadian system is photosensitive to light only during the subjective night, then perhaps this was true for reproductive responses as well. We tested this utilizing LD_{FB} and LL; indeed an increase in the light intensity in either lighting conditions increased the occurrence of persistent estrus in the rat. In fact, in identical intensities, the development of persistent estrus was similar (Figure 8).

Light, is not only capable of changing behavioral and physiological parameters, but can also change the morphology of an organism. Testicular weight in seasonal breeders is a good example. The subjective night photosensitivity holds true for seasonal reproduction as well. If light exposure occurs during the subjective night, a long-day breeder will assume the day to be long and its reproduction function will be maintained (4, 20, 21, 40, 45, 68, 70, 80, 82). In a long day, light impinges on subjective night on both ends and shortens the duration of the well-defined melatonin peak and therefore, the animal interprets this photoperiod as a long day (the duration of elevated melatonin appears to be a photoperiod interpreter; 46, 59-63, 79). Light entrains the circadian rhythm of melatonin; light also has the circadian independent inhibition of melatonin (5, 6, 63, 64). Light exposure during the subjective night, when melatonin is normally high, will cause a fall in the peak melatonin levels, irregardless of the circadian period. If melatonin durations are short (the peaks can be shortened by breaking the peak into two or attenuating just one side or the other) the organism will interpret the photoperiod as a long day (17-19, 22, 65).

Constant light suppresses melatonin levels and thus, is interpreted as a long day; therefore, Syrian hamsters (which are long day breeders) are reproductively maintained in LL (i.e., large testes and sex accessory glands, high serum hormone concentrations of LH, FSH, PRL and T). Following the logic of preceding experiments, we hypothesized that LD_{FB} would also suppress melatonin production and therefore, maintain reproductive function in long day seasonal breeders such as the Syrian hamster. In an effort to test this hypothesis, we exposed animals to long photoperiods (LD14:10), short photoperiods (LD10:14), LL, DD, LD_{FB}, rLD_{FB}, LD_{FB} NC, LD1:1 and LD1m:1m. There was a significant decrease in the level of reproductive maintenance in the LD_{FB} and LD1m:1m (and LD10:14 or DD) hamsters when compared to the LL, rLD_{FB}, LD_{FB} NC, LD1:1 or LD14:10 animals, despite these animals getting as much as five to six hours of light during the subjective night (Figure 9; 28-30, 49). These results were contradictory to our understanding of the impact of nocturnal light exposure and its effect on seasonal reproduction. Apparently, the way an organism interprets light is very different than previously thought. Even more surprising, some animals in LD_{FB} who were taken out of their cage when they were running (and therefore, had the light on), decapitated immediately and had their pineals removed, were found to have high melatonin concentrations. Thus, the circadian independent inhibition of melatonin by light apparently wasn't occurring under these conditions. In fact, the waveform of melatonin in these animals appears to be normal (C.E. McCormack, personal communication). Why these organisms are not responding to light at night is something that is not yet understood; however, the effects of LD_{FB} have been verified in the female Syrian hamster (31).

Perhaps the animal's self-exposure to light is detrimental to the reproductive system because it is stressful. Measurements have demonstrated that animals in LL have larger adrenal glands, suggesting that this may be the most stressful condition for a nocturnal rodent. Exposing

the animals to the opposite condition, rLD_{FB} , where a hamster turns the light off when it runs, instead of turning it on, receives most of its light during the subjective day. The activity pattern of hamsters, however, is bimodal; therefore, there is substantial light exposure during the subjective night. In this lighting condition, animals are maintained. Self-controlled light, therefore, is not the problem with maintenance.

$LD_{FB} NC$, a condition in which the animal has no control over the light, but receives the same light pattern as a randomly paired animal in LD_{FB} (who is controlling both animals lighting) is also capable of maintaining normal reproductive function. The difference is that the $LD_{FB} NC$ animal can assume any phase relationship with the light, whereas the LD_{FB} animal is phase locked into having light during the subjective night. Light exposes the $LD_{FB} NC$ animal's subjective night frequently (every 4 or 5 days). The pattern of light, therefore, is not the photoinhibitory effect of LD_{FB} (it's not the duration of light, nor is it the pulsital nature of the light). The results for testicular weight, parallel the lighting effects on serum hormone concentrations of T, LH, FSH and PRL, (see Figure 10) as well as sex accessory glands, seminiferous tubule diameter, etc.

We also looked at the development in the Djungarian hamster. Unlike the Syrian hamster, the reproductive development into the first breeding season is also dependent upon the length of the photoperiod (maturation in the Syrian hamster is photoindependent; 11, 21, 47, 48, 82). Although the development of reproduction function in the Djungarian hamster is significantly stimulated by LD_{FB} , the stimulation is less effective than LL or long photoperiods (Figure 11; 26, 27). These results further suggest that there is definitely some misunderstanding in our perception of the way that animals interpret light.

The effect of light on the reproductive systems of seasonal breeders is even further complicated by results in the adult Djungarian hamster (77). Mature Djungarian hamsters are maintained by LD_{FB} (Figure 12). The mature Djungarian hamster must, therefore, interpret light very differently than the Syrian hamsters does, despite the fact that the PRCs are very similar. Perhaps the response of melatonin to light might be species (and development) specific.

It was previously demonstrated by phase-response curves that most mammals and some other species are photosensitive during the subjective night and insensitive to light during the subjective day; furthermore, the length of the period of the endogenous rhythm, in constant conditions, is a function of the intensity of the light. Organisms are also differentially sensitive to different wavelengths of light. The Syrian hamster is most sensitive to wavelengths of approximately 500 nanometers (78). Furthermore, an animal can phase shift to a greater extent with the same energy output, provided the appropriate wavelength is used. This concept may be a very important point to a group such as NASA, where important considerations on the shuttle are weight, energy and crew time. This is a way to use less energy and produce the same effects.

Other organisms have very similar action spectrums. For example, the most sensitive wavelength for a fruit fly is also very close to 500 nanometers, as are the action spectrums for the finch, the lizard and the human (16). All of these curves are similar to the rhodopsin sensitivity curves. Rhodopsin is generally found in rods, but has also been suggested as being in other components such as the ganglion cells. This may also be an important point, since rods may not be needed for photoreception in the circadian or photoperiodic system. Bright constant light has been implicated in rod degeneration. These animals apparently have diminished visual discrimination and yet respond very well to light and can entrain to light cycles. Eyes *per se* are not even necessary. *Neurospora* and *Gonyaulax* do not have eyes; yet both have circadian rhythms, respond to light, have PRCs and spectral sensitivity curves very similar to other organisms with eyes (42, 66, 67). *Paramecium* also possesses circadian rhythms, but the peak wavelength is closer to 6-700 nanometers; however, there is also a peak close to 500 nanometers which might be responding in a circadian fashion (66). The stronger red response in *Paramecium* is not completely understood.

Therefore, if an experimenter needs visual observations of an animal's cage in the middeck, a Kodak filter (which is 99.99% dense below 610 nanometers) will eliminate most, if not all, of the effects that the light will have on the circadian and reproductive systems. Opening the middeck cage door without a filter, without a hood or something to restrict the light entering that cage, the animals will respond to the 250 lux in the middeck (personal communication, C. Wheelwright; JSC-NASA), while virtually ignoring the 30 lux inside the cage. This will cause profound and confusing effects.

Light can also complicate the effects of other modulators. In a previous experiment, the question was asked whether circadian rhythms can persist outside the geophysical rhythms of the earth's environment (7, 8). To answer this, *Neurospora crassa* grown in "race" tubes were flown aboard shuttle flight SL-1 (74, 75). In the dark, on a specific media, the *Neurospora* conidiation banding occurs at approximately 21.5 - 22 hours intervals. In constant light, conidiation is arrhythmic. Seven days into the flight, the astronaut took the package out of constant dark and exposed them to the middeck lighting in order to mark the growth fronts. Prior to the light exposure, banding had occurred with a decreased amplitude and in some tubes the banding was completely damped (i.e., arrhythmic). Following the crew marking procedure rhythmicity was reinstated. The cultures remained rhythmic for the remainder of the flight. It was hypothesized that the difference between the damped banding before the light pulse and the robust banding following the light pulse may have been the hypergravity pulse encountered during launch. Light exposure, apparently, reversed the effect of a hypergravity pulse (50).

In order to test this hypothesis, we exposed *Neurospora* to hypergravity through acute and chronic centrifugation. Chronic hypergravity (3 G for 7 days) increased the period of the circadian rhythms of conidiation. This increase in period length appeared to persist and continue to increase throughout the experimental exposure. Biological rhythms of mammals are also affected by hypergravity (38, 39). The increase in period was very similar to the effects seen in

monkeys and rats. Chronic hypergravity, however, did not damp the circadian rhythmicity in *Neurospora*. Acute 3 G (10 minute) exposure did not significantly lengthen the period, however, damping occurred, especially in band strain (Figure 13). Exposing these tubes to light eliminated the damping effect of acute hypergravity in this organism. Therefore, there are far ranging effects of light, especially on the circadian system. Since, the circadian system affects almost every system in eukaryotes, the effects of light have to be considered in all experiments.

Finally, there appears to be a hypothermia response in the microgravity environment of space. Rhesus monkey axillary (core) temperature was measured on Cosmos flight 1514 (76). Ankle temperature was also measured and found to be reduced in space (probably due to vasoconstriction) indicating a minimal heat loss. Hypo-thermia, therefore, was probably not due to an increased heat loss. Heart rate data suggest that the hypothermia may be due to a decrease in the metabolic rate. Several experiments have suggested a decreased metabolic activity in space. This result may suggest that other physiological functions are altered in the microgravity environment of space, including effects on photoreception and circadian rhythmicity.

Discussion

Most eukaryotic organisms possess endogenous oscillators. These oscillators control many, if not most, of the physiological and behavioral functions in these organisms. Most eukaryotic organisms also respond similarly to light, with phase delays in the early subjective night, phase advances in the late subjective night and a relative insensitivity to light during the subjective day. Furthermore, abbreviated lighting conditions can produce many of the effects of longer pulses and/or constant light.

Our lighting recommendations to NASA would include the use of a full spectrum light in both experimental paradigms and crew quarters. With respect to circadian rhythms, however, a light source with a narrow band around 500 nanometers would be more than sufficient if energy is in short supply. Other spectral bands, however, are important for other concerns, such as vitamin D; therefore, a full spectrum may be more appropriate. Consistency in the lighting conditions between experiments should be encouraged. Minimal lighting differences between the experimental lockers and the middeck should also be encouraged. A mechanism to observe experiments without changing the lighting conditions (light exposure from the middeck) should be developed. Timer controlled and constant lighting (including constant dark) conditions should be available to NASA scientists. Light sources with minimal heat radiation are preferable, despite the temperature compensated systems of biological clocks. Experimental lockers should be light-tight and well ventilated, even when the experimental conditions require light cycles.

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Table 1

| Species | Lighting Condition | | | | Differences | | |
|----------------------------------|---------------------|--------------------|--------------------|-------------------|-------------|----------------------|----------------------|
| | Free-running Period | | | | LL-DD | LD _{FB} -DD | LD _{FB} -LL |
| | DD | LD _{FB} | LL | D : A | | | |
| Rat* (F) | 24.01±0.02 (66) | 25.54±0.07 (27) | 25.45±0.11 (8) | 1.50 [@] | 1.35 | 1.44 | 0.09 |
| Syrian Hamster [†] (F) | 24.06±0.01 (6) | 24.84±0.04 (6) | 24.72±0.11 (6) | 0.53 [©] | 0.78 | 0.66 | 0.12 |
| Syrian Hamster [†] (M) | 24.22±0.06 (8) | 24.60±0.07 (5) | 24.57±0.06 (9) | 0.53 [©] | 0.35 | 0.38 | 0.03 |
| Syrian Hamster [§] (M) | 24.16±0.04 (16) | 24.73±0.03 (15) | 24.56±0.06 (8) | 0.53 [©] | 0.40 | 0.57 | 0.17 |
| Djung. Hamster (M) | 23.99±0.05 (15) | 24.59±0.10 (12) | 24.70±0.10 (12) | 1.04 [®] | 0.71 | 0.60 | -0.11 |
| Mouse [¥] (F) | 23.82±0.06 (15) | 25.09±0.20 (8) | 24.78±0.05 (6) | 4.30 [©] | 0.96 | 1.27 | 0.31 |
| Squirrel Monkey [™] (M) | 24.5±0.1 (9) | 25.8±0.1 (8) | 25.6±0.1 (11) | 0.53 [¶] | 1.10 | 1.30 | 0.20 |

* Ferraro and McCormack, 1984; 1986

@ Summer *et al.*, 1984

† Ferraro and McCormack, 1985

© Pittendrigh and Daan, 1976b

® Milette and Turek, 1986

¥ Ferraro, 1984

™ Ferraro and Sulzman, 1988

¶ Hoban and Sulzman, 1985

§ Ferraro *et al.*, 1987

(F) = females

(M) = males

(#) = number of animals per group

Period data given in hours as Mean ± SEM

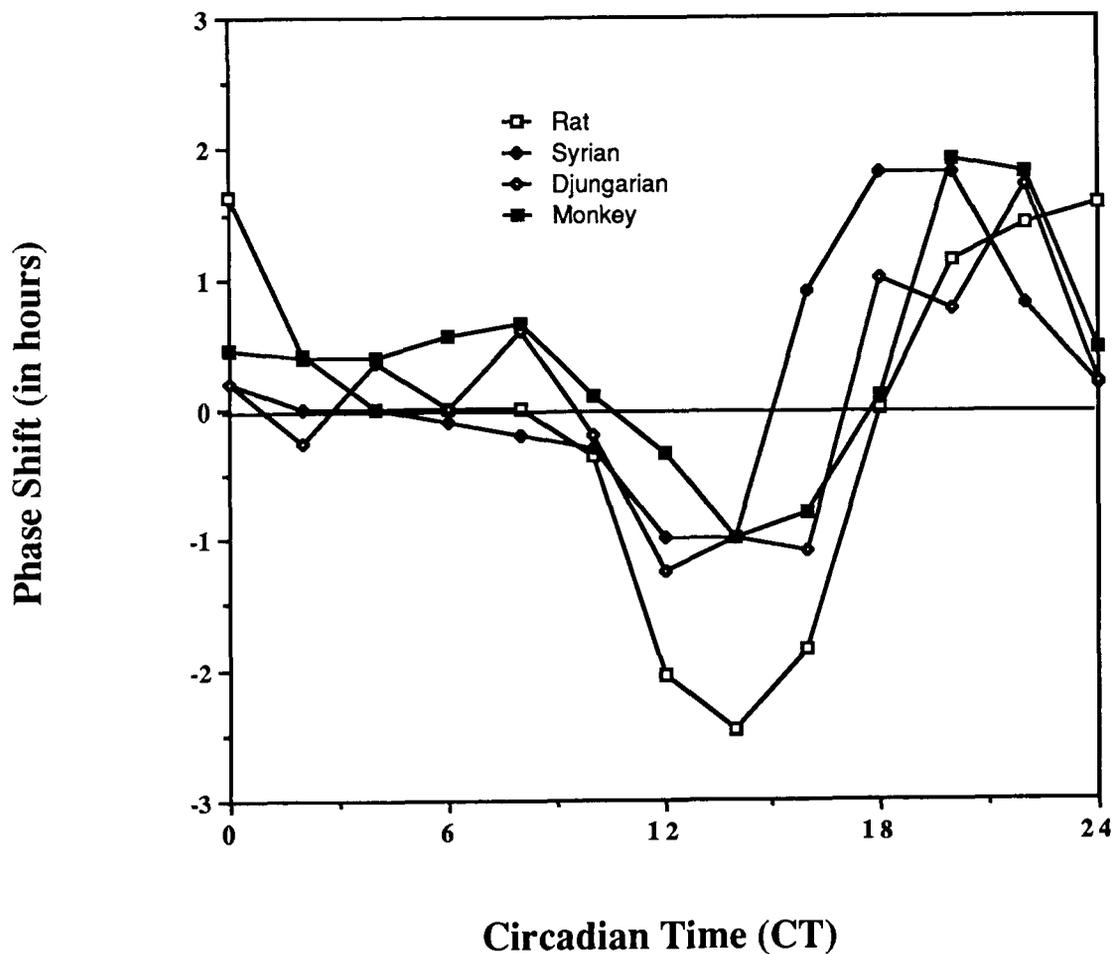


Figure 1. Phase-response curves (PRC) for the rat (Summer *et al.*, 1984), Syrian hamster (Daan and Pittendrigh, 1976a), Djungarian hamster (Milette and Turek, 1986) and squirrel monkey (Hoban and Sulzman, 1985) are plotted in circadian hours (CT; i.e., one circadian cycle is divided into 24 equal circadian hours); CT12 usually represents activity onset, and therefore the beginning of the subjective night, in nocturnal rodents. PRCs are graphic representations of the photosensitivity of a given circadian oscillator as determined by light perturbation administered at different phases of the circadian cycle. In each of these mammals, the photosensitivity of the circadian oscillator to light is similar; phase delays produced by light exposure during the early subjective night, phase advances resulting from exposure to light during the late subjective night and relative insensitivity during the subjective day. This response to light is a common feature of the circadian oscillator; similarly shaped PRCs have been developed for lower vertebrates, invertebrates and even single cell organisms.

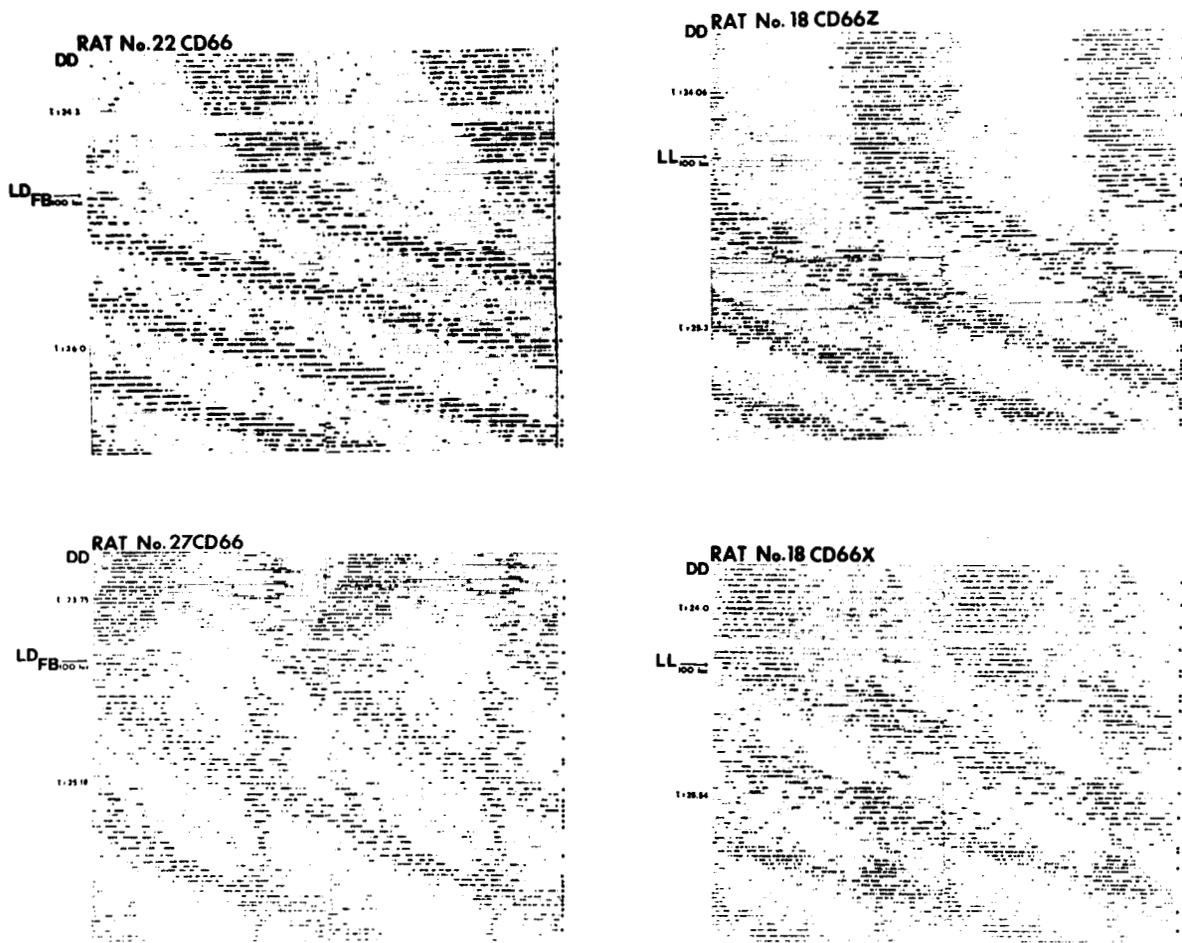


Figure 2. Double-plotted actographs of 4 rats {2 exposed to 100 lux feedback lighting (LD_{FB}) and 2 to 100 lux constant light (LL)} demonstrate similar free-running activity rhythms. Free-running periods and lighting conditions are given in the left margin; vaginal smear records are denoted in the right margin (closed circles denote days of estrous type vaginal smears). Rats 27CD66 and 18CD66Z lost their ovulatory cyclicity, whereas rats 22CD66 and 18CD66X did not. DD, continuous darkness. (33)

PS12

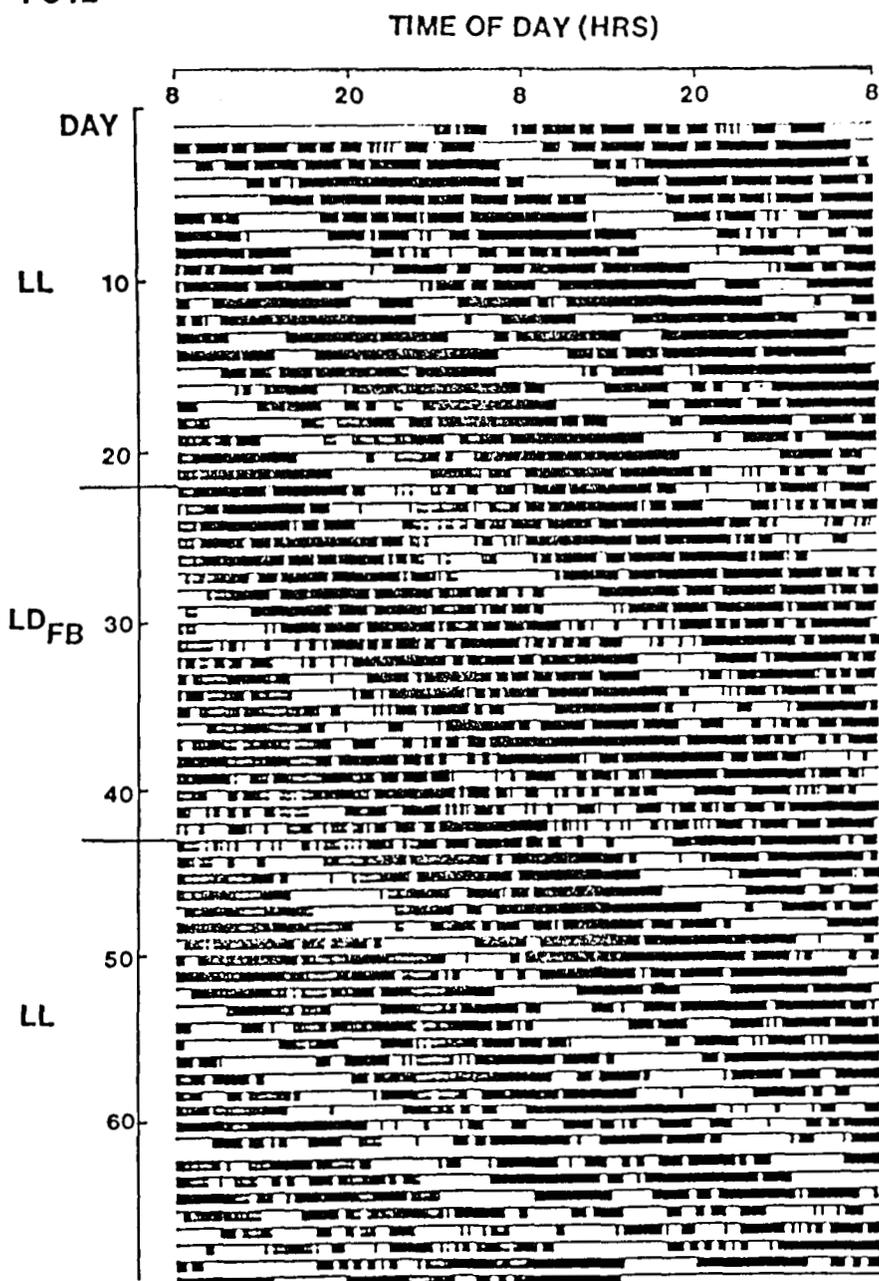


Figure 3. Double-plotted actograph of a squirrel monkey demonstrating similar free-running periods in constant light (LL) and feedback lighting (LD_{FB}). Lighting conditions and day number are given in the left margin; 48 consecutive hours are represented across the top of the actograph. The free-running period during the second exposure to LL starts from the predicted phase, demonstrating a lack of masking in LD_{FB}. (35)

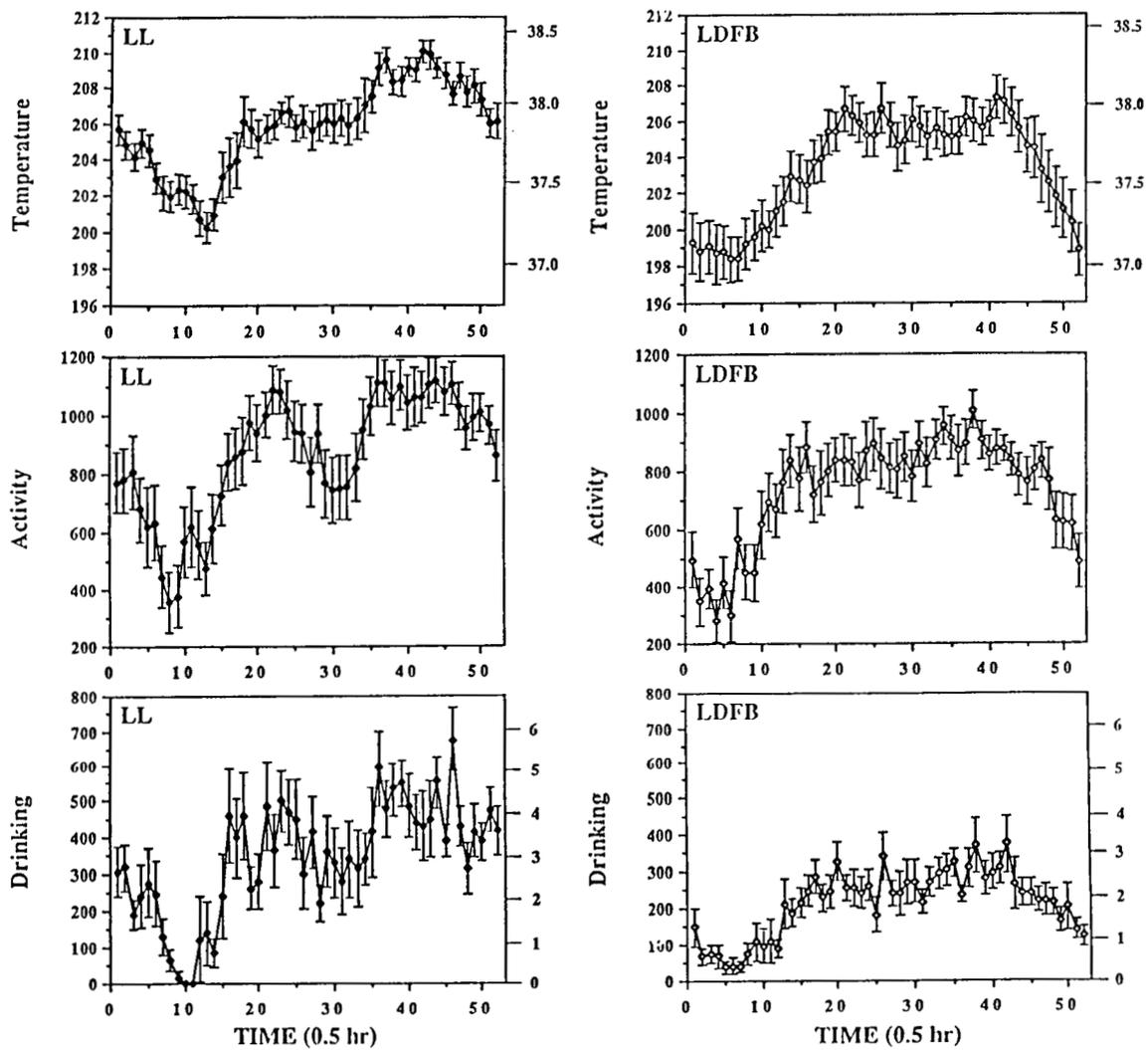


Figure 4. Educued (average) waveforms of three simultaneously recorded circadian rhythms (core body temperature, locomotor activity and drinking) of a squirrel monkey in constant light (LL) and feedback lighting (LD_{FB}) demonstrates a similar phase relationships between these oscillations in both lighting conditions. The level of the given activity is denoted in the left margin (corrected temperature, in $^{\circ}C$, and drinking volume, in millileters, is given in the right margin); time is represented across the bottom of each waveform. (35)

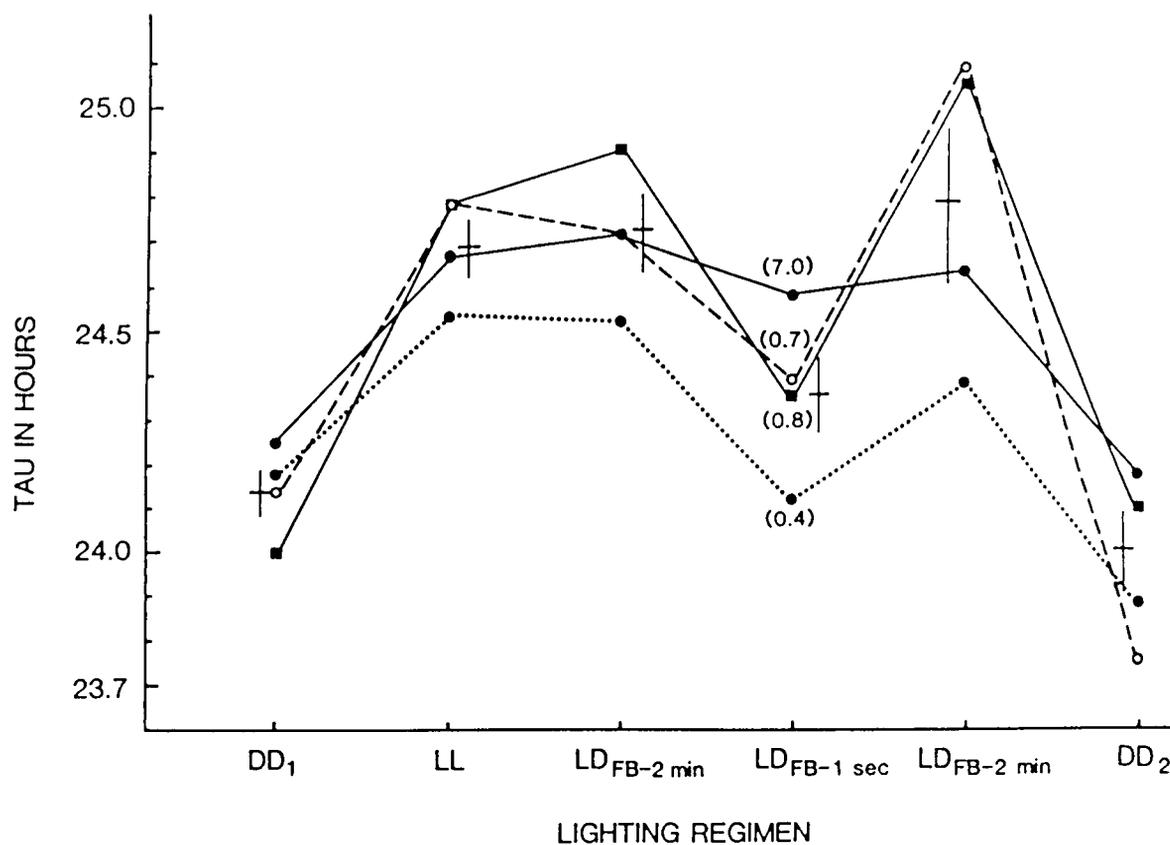


Figure 5. Effect of experimental lighting on the free-running period (τ) in four individual rats. The numbers next to each curve above LD_{FB} 1 sec are the actual measured light pulse durations. The short horizontal line above each regime represents the mean τ for that condition \pm SEM (vertical bar). For all rats, except the one in which the light pulse was 7 seconds in duration, τ shortened considerably when the LD_{FB} exposure duration was switched from 2 minutes to 1 second, and lengthened again when the animal was returned to LD_{FB} 2 min. Mean taus in LL did not differ from the mean taus in either exposure of LD_{FB} 2 min; however, exposure to LD_{FB} 1 sec significantly shortend τ ($P < 0.05$). (32)

Period-Intensity Relationship

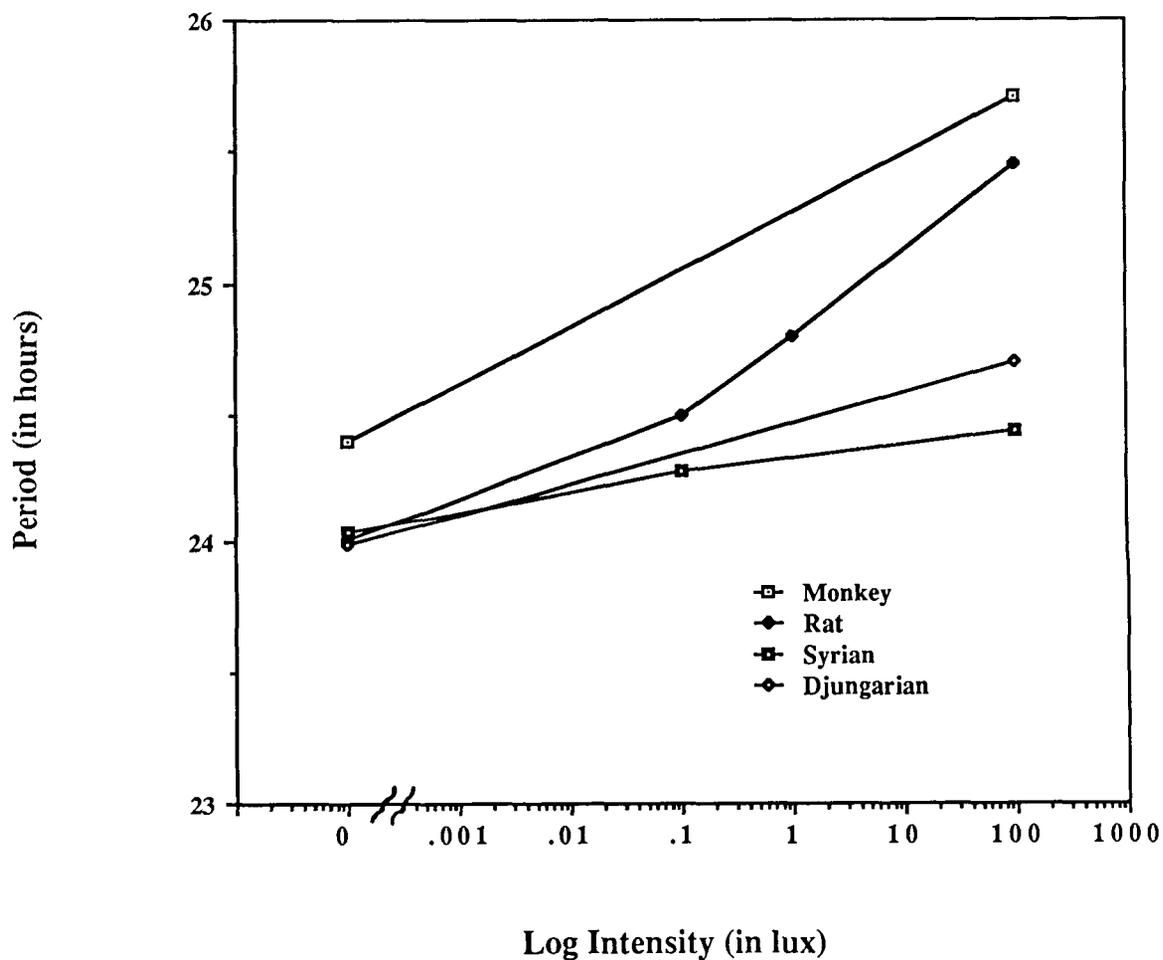


Figure 6. Aschoff-Intensity curves for the rat (Summer *et al.*, 1984), Syrian hamster (Daan and Pittendrigh, 1976a), Djungarian hamster (Ferraro, unpublished) and squirrel monkey (Hoban and Sulzman, 1985) are plotted in the log ($\times 100$) of the lighting intensity. In each of these mammals, as the intensity of the light increases, the length of the free-running circadian period increases. This response to light is a common feature of the circadian oscillators of most nocturnal and some diurnal species.

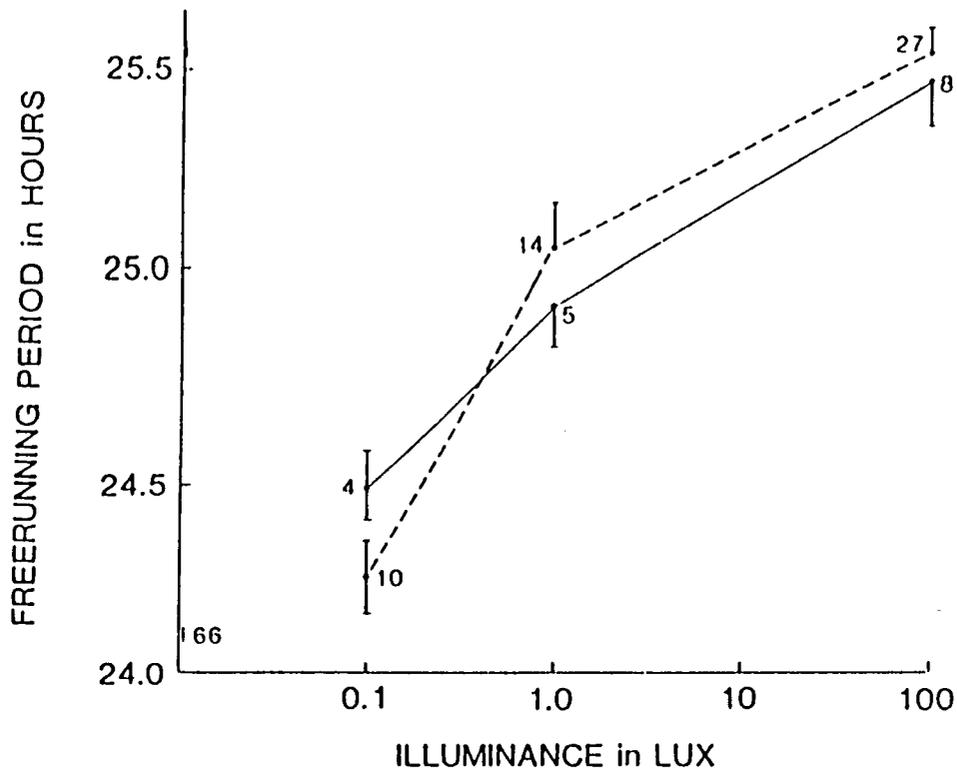


Figure 7. Aschoff-Illuminance curves for rats exposed to three different intensities of constant light (LL; solid line) of feedback lighting (LD_{FB}; dotted line). The log of the light intensity is plotted against the mean free-running period (τ). The number of animals on which the mean is based is given next to the mean. The mean τ for rats (N=66) in constant darkness is given in the lower left corner. For both LL and LD_{FB} groups, τ increased significantly ($P < 0.05$) with each increase in intensity. The mean τ s for animals exposed to LL or LD_{FB} did not differ significantly at any intensity. (32)

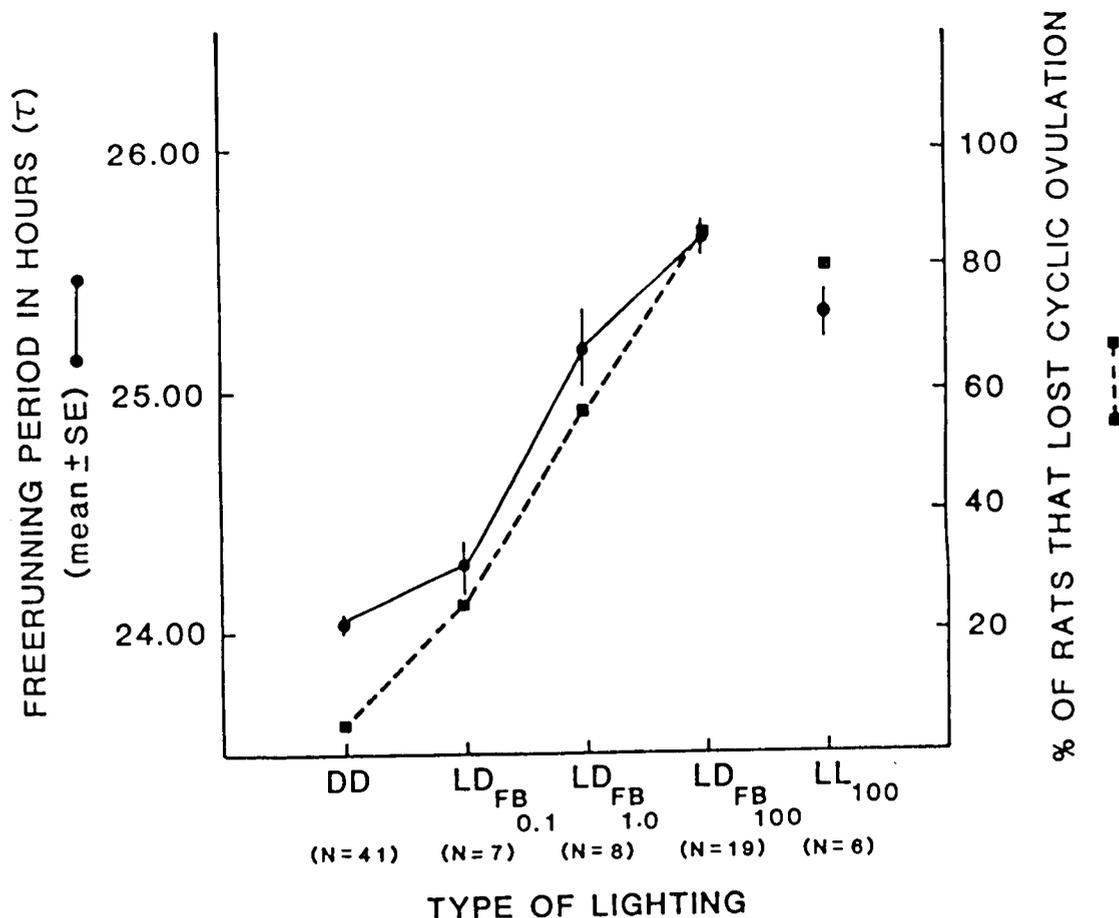


Figure 8. Effects of increasing intensity of feedback lighting (LD_{FB}) on the free-running period (τ ; solid line) of locomotor activity and the loss of cyclic ovulation (dotted line) in the albino rat. Vertical lines on the curve depicting τ represent the SEM. The number of rats in each condition are given by N on the abscissa. Increasing intensities of LD_{FB} increase τ and the proportion of rats that lost cyclic ovulation. At an intensity of 100 lux, the reproductive and circadian effects were similar in LD_{FB} or constant light (LL). (33)

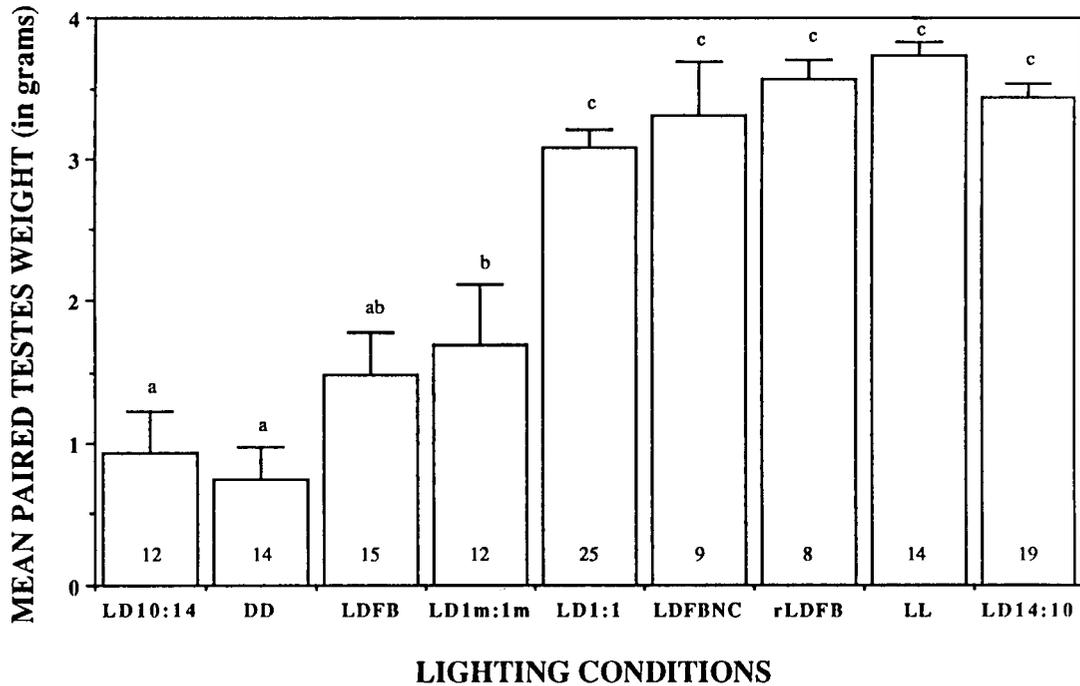


Figure 9. Mean paired testes weights of Syrian hamsters exposed for ten weeks to one of the following conditions: constant dark (DD), a short photoperiod (LD10:14), feedback lighting (LD_{FB}), an ultra-short light-dark cycle (LD1m:1m), a short-light dark cycle (LD1:1), feedback lighting neighbor control (LD_{FB} NC), reverse feedback lighting (rLD_{FB}), constant light (LL) and a long photoperiod (LD14:10). Standard error of the mean (SEM) and number of animals per group are also given (above the bars and at the bottom of the bars, respectively). Means with dissimilar letters above the SEM bars were significantly different. Animals exposed to DD, LD10:14, LD_{FB} and LD1m:1m do not have maintained testes, while animals exposed to LD1:1, LD_{FB} NC, rLD_{FB}, LL and LD14:10 do.

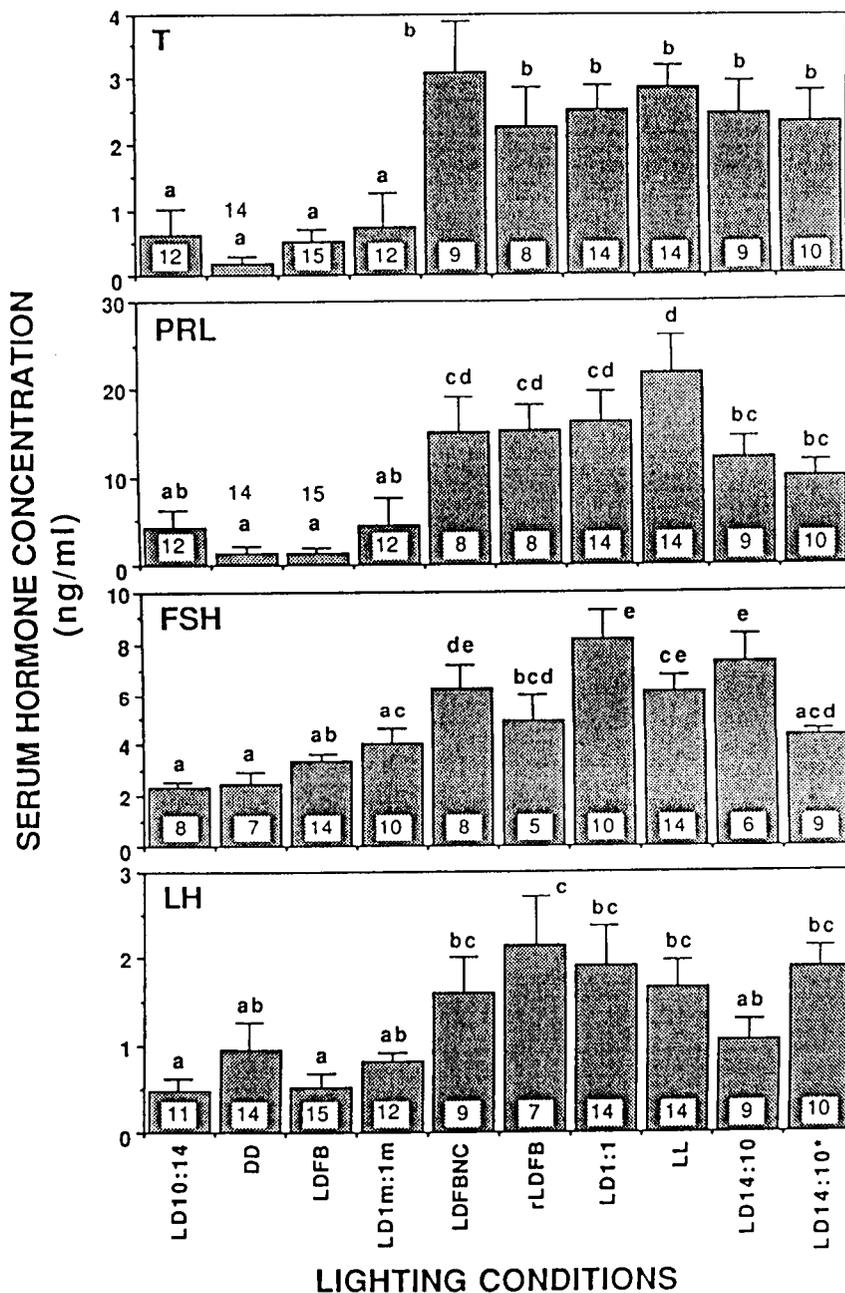


Figure 10. Serum hormone levels of testosterone (T), prolactin (PRL), follicle stimulating hormone (FSH) and luteinizing hormone (LH) in ng/ml of Syrian hamsters exposed for ten weeks to one of the following conditions: LD10:14, DD, LD_{FB}, LD1m:1m, LD_{FB} NC, rLD_{FB}, LD1:1, LL and LD14:10 with and LD14:10* without wheels. Means with similar superscripts are not significantly different. Animals exposed to DD, LD10:14, LD_{FB} and LD1m:1m have low serum hormone levels, while animals exposed to LD1:1, LD_{FB} NC, rLD_{FB}, LL and LD14:10 have high serum hormone levels.

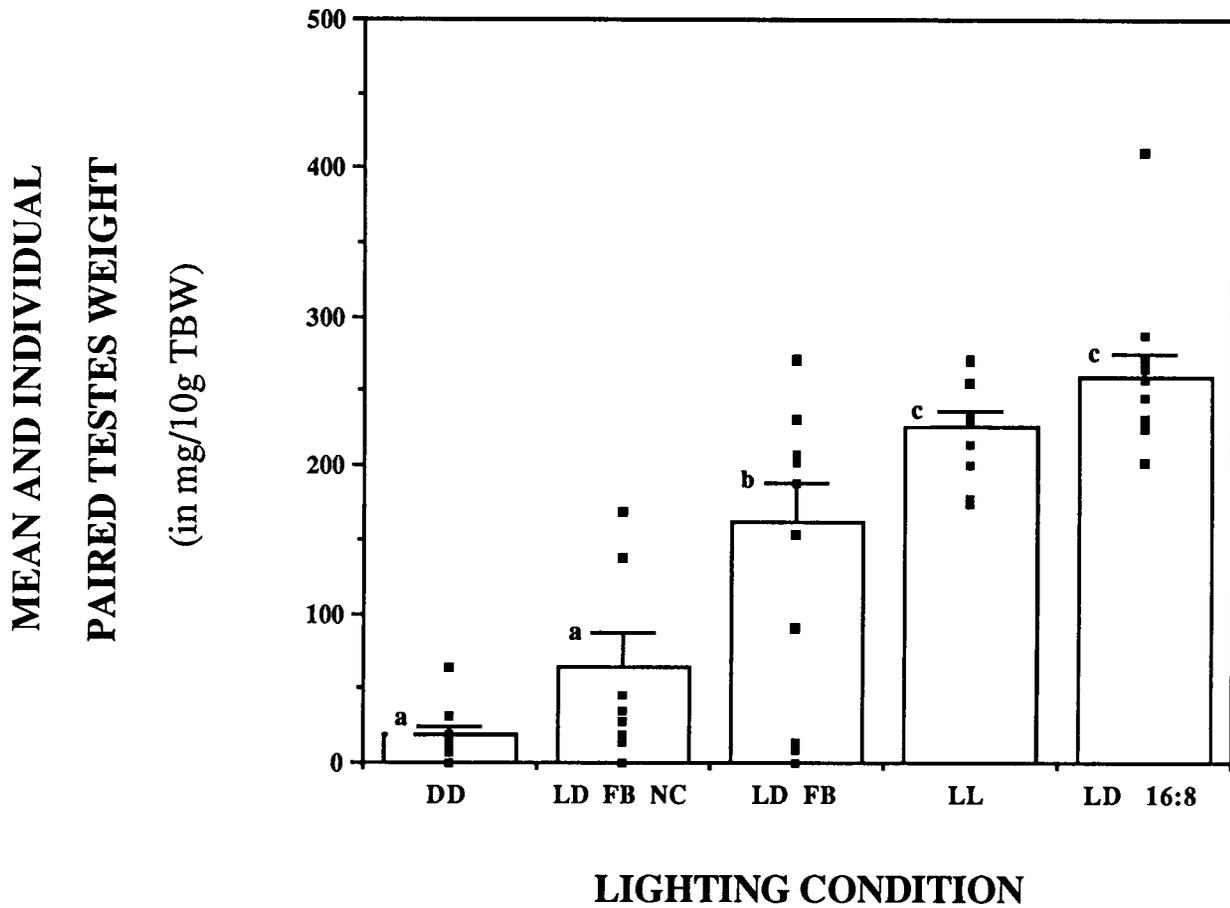


Figure 11. Mean and individual paired testes weights (mg/10g total body weight) of immature Djungarian hamsters exposed for 30 days to one of the following conditions: constant dark (DD), feedback lighting (LD_{FB}), feedback lighting neighbor control ($LD_{FB} NC$), constant light (LL) and a long photoperiod ($LD_{16:8}$). Standard error of the mean are given above the bars, while individual data are presented as filled boxes. Means with similar superscripts are not significantly different. Animals exposed to DD or $LD_{FB} NC$ have a significantly slower reproductive development than animals exposed to LD_{FB} , LL or $LD_{16:8}$. Animals exposed to LD_{FB} , while developing more rapidly than animals in DD or $LD_{FB} NC$, develop slower than animals in LL or $LD_{16:8}$. (26)

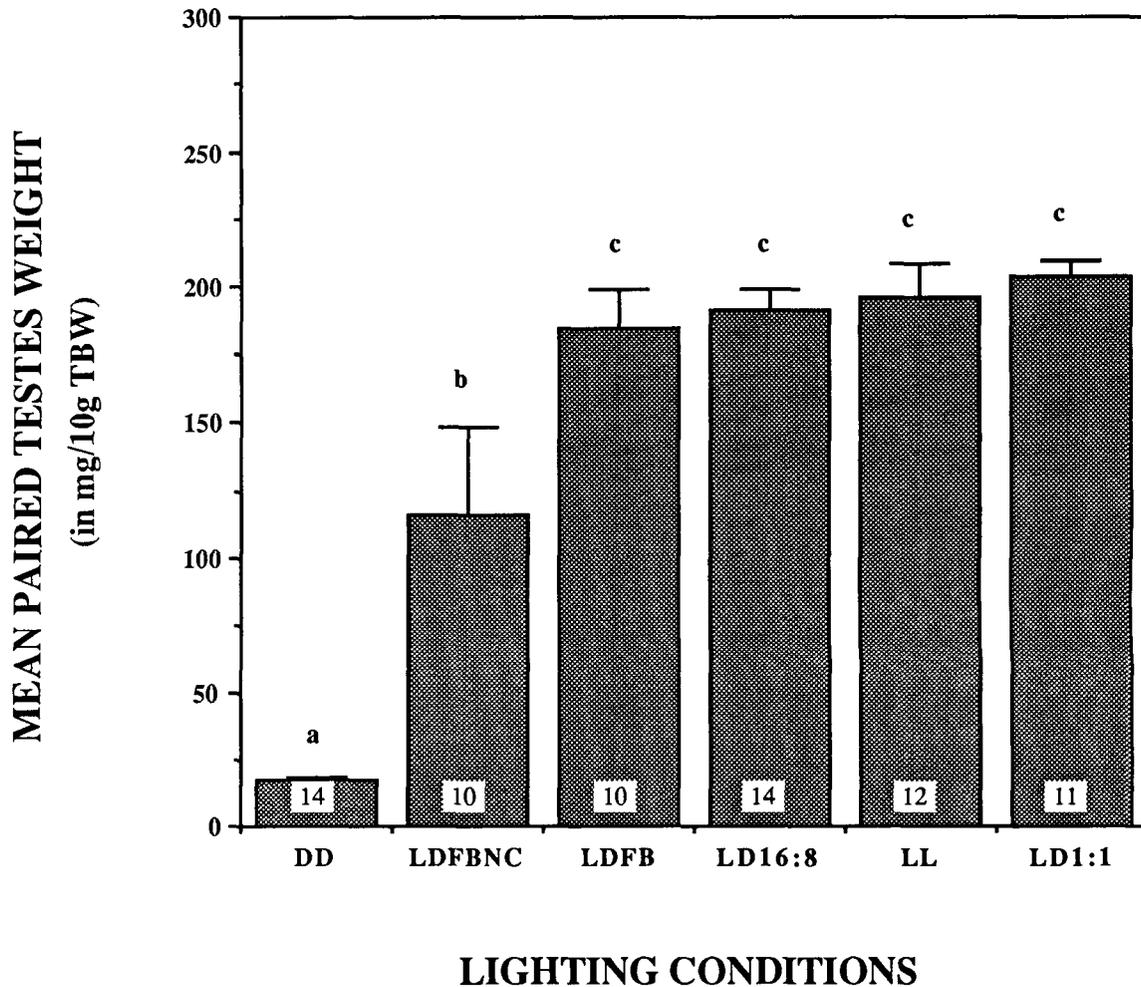


Figure 12. Mean paired testes weights (mg/10g total body weight) of mature Djungarian hamsters exposed to one of the following conditions: constant dark (DD), feedback lighting (LD_{FB}), feedback lighting neighbor control ($LD_{FB} NC$), constant light (LL), a short light-dark cycle (LD1:1) and a long photoperiod (LD16:8). Standard error of the mean are given above the bars. Means with similar superscripts are not significantly different. Animals exposed to DD or $LD_{FB} NC$ have a significantly slower reproductive development than animals exposed to LD_{FB} , LL, LD1:1 or LD16:8. Animals exposed to LD_{FB} are as maintained reproductively as animals in LL, LD1:1 or LD16:8.

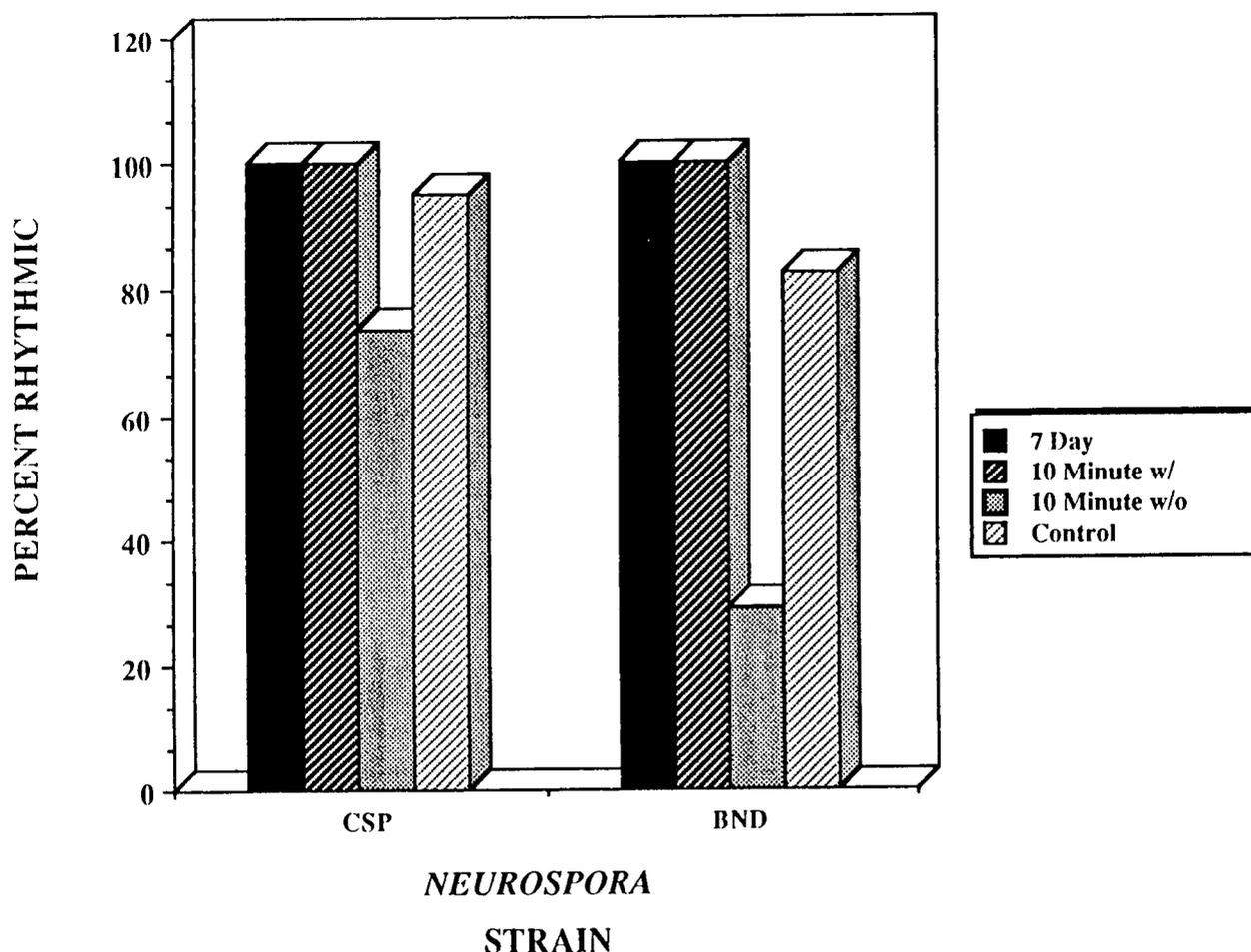
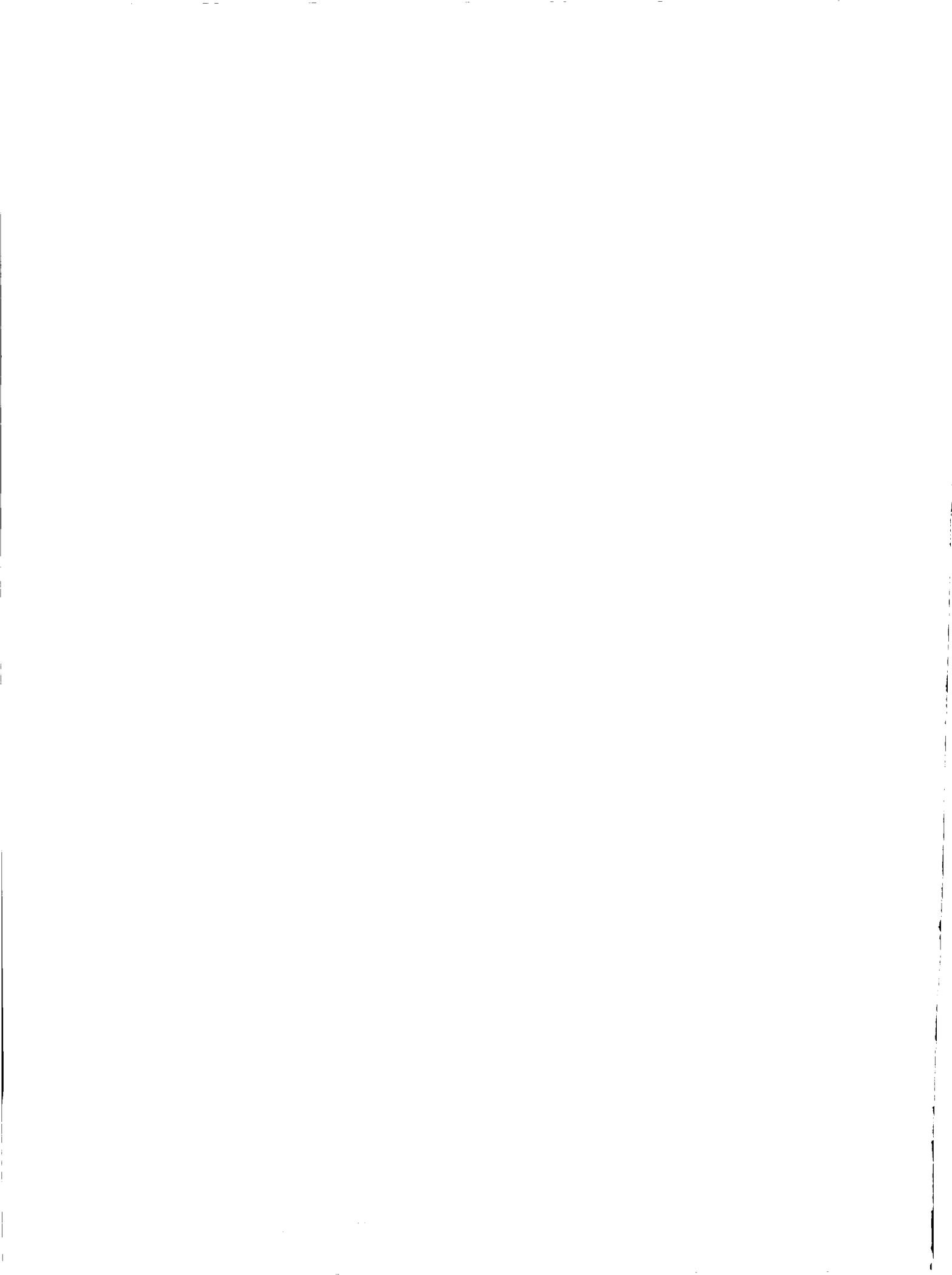


Figure 13. Effects of chronic (7 day) and acute (10 minute) exposure of hypergravity (3 G) on the damping of the conidiation rhythm in two strains (BND and CSP) of *Neurospora crassa*. While chronic exposure produces increases in the free-running period of the rhythm (see text), it has no effect on rhythm damping. Contrarily, acute exposure to a hypergravity pulse, has little effect on the free-running period, but produces pronounced damping (10 Minute w/o). This damping effect of acute hypergravity can be eliminated with a brief light exposure (10 Minute w/). The controls displayed minor damping. This is quite unusual, however, and in repeat experiments was not seen, while acute hypergravity repeatedly caused damping and light pulses repeatedly reversed the effect.



ILLUMINATION OF ANIMAL QUARTERS IN MICROGRAVITY HABITATS:
PARTICIPATION OF LIGHT IRRADIANCE AND WAVELENGTH
IN THE PHOTIC REGULATION OF THE NEUROENDOCRINE SYSTEM

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Bold-face sections of the following manuscript highlight specific design and engineering suggestions for the construction of animal habitats aboard space craft.

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INTRODUCTION

To design optimum illumination of animal quarters aboard space vehicles or orbiting space stations, a variety of physiological considerations must be taken into account. It is well known that light is the energy which stimulates visual function. More recently, there has been a growing appreciation that light has a broader biological impact. Specifically, light regulates dermal physiology, circadian rhythms and neuroendocrine function of animals and humans (1,43,49,50). Furthermore, the regulatory capacity of light depends on its intensity and wavelength (15,49,50). Consequently, design of animal housing facilities must provide photic stimuli which are adequate for vision as well as dermal, circadian and neuroendocrine regulation. Additionally, animal quarters must be sufficiently illuminated for routine maintenance and observation by astronauts and scientists. Such design considerations are equally valid for both space and ground-based animal facilities. The following paper will review data pertinent to the impact of the physical parameters of light which regulate the neuroendocrine systems of rodents and primates.

EFFECT OF LIGHT IRRADIANCE ON MELATONIN

The hamster is a nocturnal burrowing rodent. During the day when the planet surface illumination is high, the hamster is predominantly in darkness in its burrow. However, rodents emerge briefly from their burrows and expose themselves to pulses of environmental light throughout the day (27,36,37). When the

sun sets, the hamster begins its primary activity period. It emerges from its burrow and is active primarily during the nocturnal hours when the planet surface is dark.

Along with this circadian behavioral pattern there are internal physiological and biochemical rhythms. One biochemical rhythm of interest is the rhythmic production of the hormone melatonin from the pineal gland. It is well established that light stimulates the mammalian retina, thus sending signals from the retina to the suprachiasmatic nucleus (SCN), an endogenous oscillator or the putative biological clock in the hypothalamus (24,30,31). The SCN relays photic information to a variety of structures in the brain. One of the best characterized efferent projections from the SCN is the pathway to the pineal gland. Photic information is relayed from the SCN to the hypothalamic paraventricular nucleus (PVN). Long projections extend from the PVN to the upper thoracic spinal cord and sympathetic fibers carry neural information from the spinal cord to the pineal gland, which entrains the circadian rhythm of melatonin production (24,30,31).

In all mammalian species studied, melatonin is secreted in a consistent daily rhythm: high melatonin levels during the night time and low melatonin levels during the daylight hours (38). Typical melatonin rhythms of hamsters which were housed either indoors or outdoors for three weeks during the winter are illustrated in Figure 1. Both sets of animals exhibit low melatonin levels during the daytime. Four to five hours after indoor lights went off or the sun set outdoors, there was a rapid

increase in synthesis of melatonin. After only 3 weeks, those animals kept out of doors had significantly higher peaks of melatonin production. This simplistic experiment was repeated the next winter with similar results: outdoor illumination and housing produced significant differences in nocturnal production of melatonin (11).

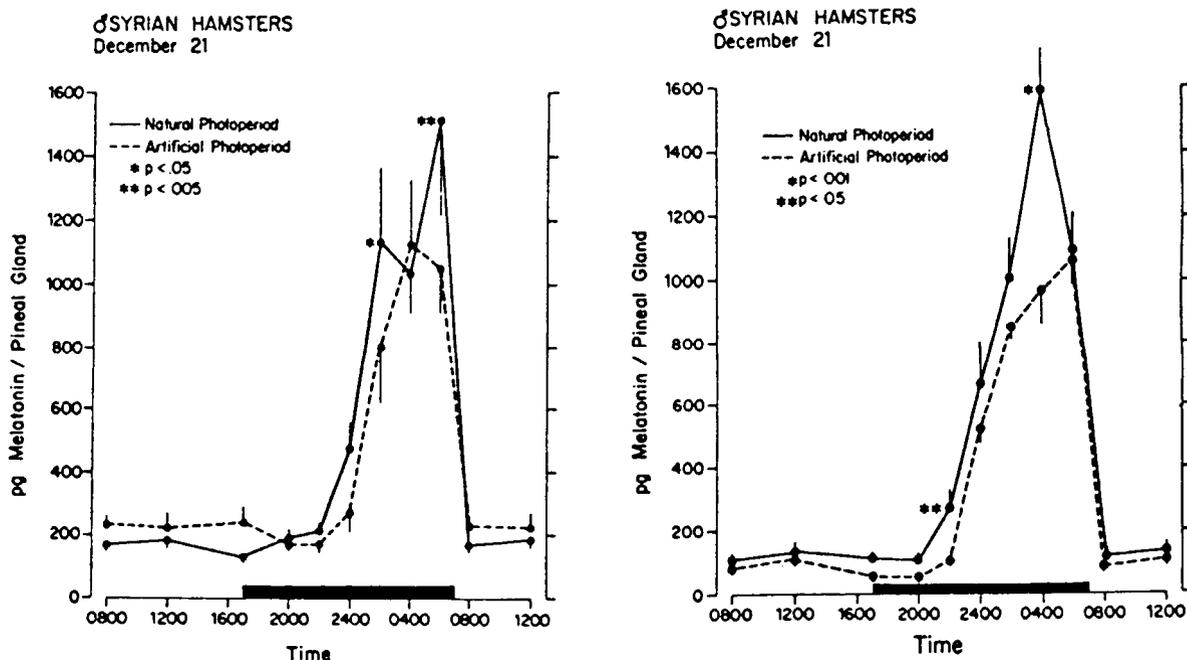


Figure 1: Melatonin rhythms in animals housed in artificial (dashed lines) versus outdoor lighting (solid lines) (11).

A variety of factors may have contributed to differences in melatonin production since animals housed outdoors are exposed to different conditions such as humidity, temperature, air ions, and smells. However, since melatonin production is dominated by the photic environment (38), it is plausible that differences in the quality of illumination produces changes in melatonin. The brightness of sunlight on the surface of the planet at midday is 50,000 uW/cm². (Table III at the end of this manuscript includes conversions of most radiometric measures to a photopic (lux)

measurement for comparative purposes). In a typical animal room, animals are exposed to 100-200 uW/cm². Similarly, there are differences in spectral quality between natural and artificial light. Sunlight has a different mixture of wavelengths compared to indoor fluorescent or incandescent lighting (45).

The studies described above led to more controlled studies on neuroendocrine effects of different physical parameters of photic input. As shown in Figure 2, photic parameters can vary in light irradiance, light wavelength, duration of light stimulus and circadian timing during which the stimulus is presented. Although this paper will focus on wavelength and irradiance control of neuroendocrine parameters, it is important to recall that duration and time of exposure are important photic parameters (1,5,22,30).

PHYSICAL PARAMETERS OF PHOTIC INPUT

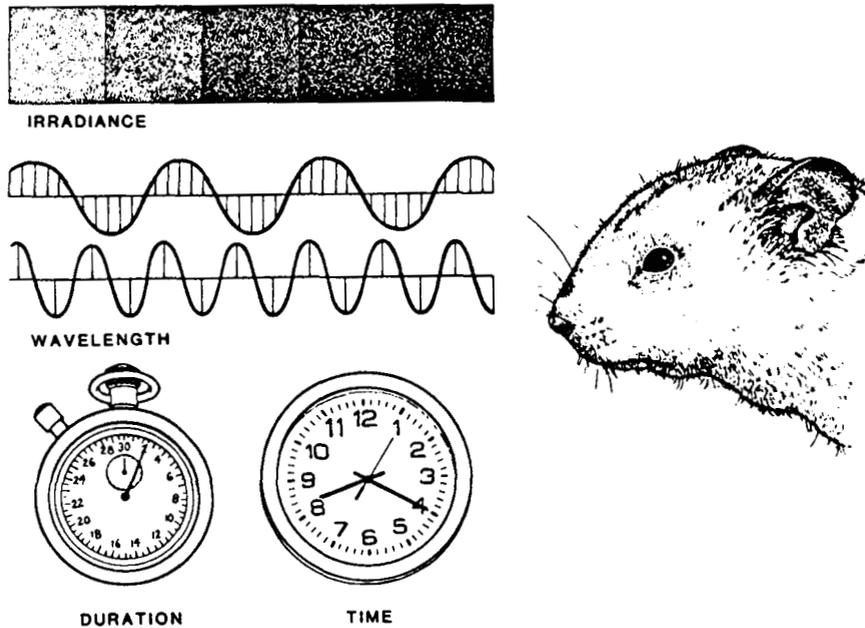


Figure 2

In addition to entraining the rhythm of melatonin, light can acutely inhibit melatonin synthesis. Specifically, during the night when melatonin is high, unexpected exposure to light induces a rapid suppression of melatonin synthesis and secretion (7,8,15,23,26,41,42). The data in Figure 3 illustrate the rapid light-induced suppression of pineal melatonin in hamsters (6). During the middle of the night, animals not exposed to light (shown in the stippled area) had high pineal melatonin contents. At time zero, animals were exposed to a high irradiance (928 uW/cm²) or a low irradiance (0.186 uW/cm²) of white light and pineals were collected 2 minutes, 8 minutes and 32 minutes after the lights were turned on. Light induced a rapid suppression of melatonin after exposure to either irradiance (6).

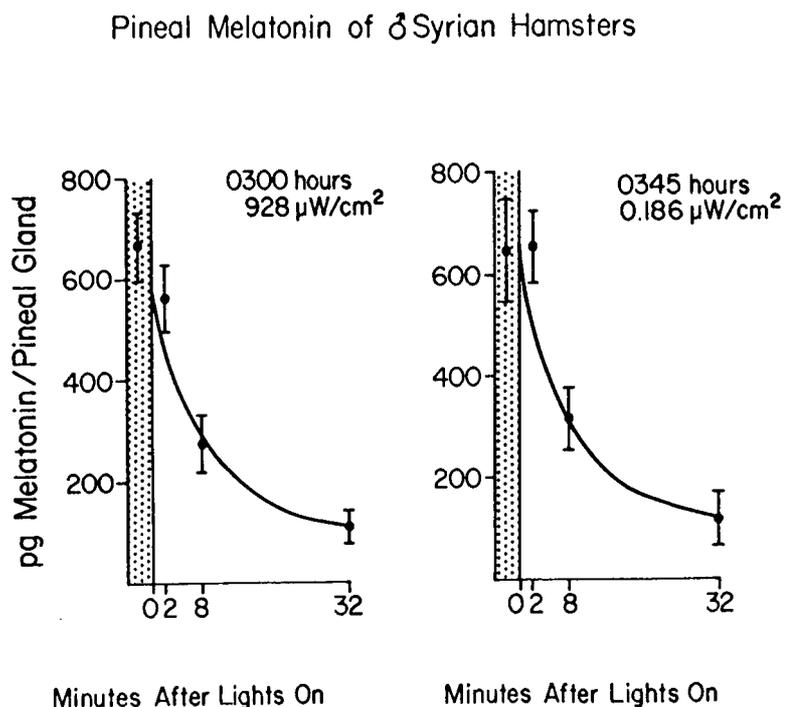


Figure 3: Rapid light-induced melatonin suppression (6).

Figure 4 illustrates a fluence-response (dose-response) curve for the suppression of melatonin by white light (8). Animals exposed to 20 minutes of low intensities (0.019 uW/cm² or lower) showed no significant suppression of melatonin. In contrast, animals exposed to 20 minutes of 0.037 or 0.074 uW/cm² of light had a mid-range melatonin suppression, and animals exposed to 0.111 uW/cm² or higher exhibited a very significant suppression of melatonin. Interestingly all light irradiances which were tested allowed both the animals and experimenter to see the environment, whereas, the low irradiances were ineffective for melatonin suppression. Hence, there is a difference in threshold sensitivity for vision versus neuroendocrine responsiveness (8).

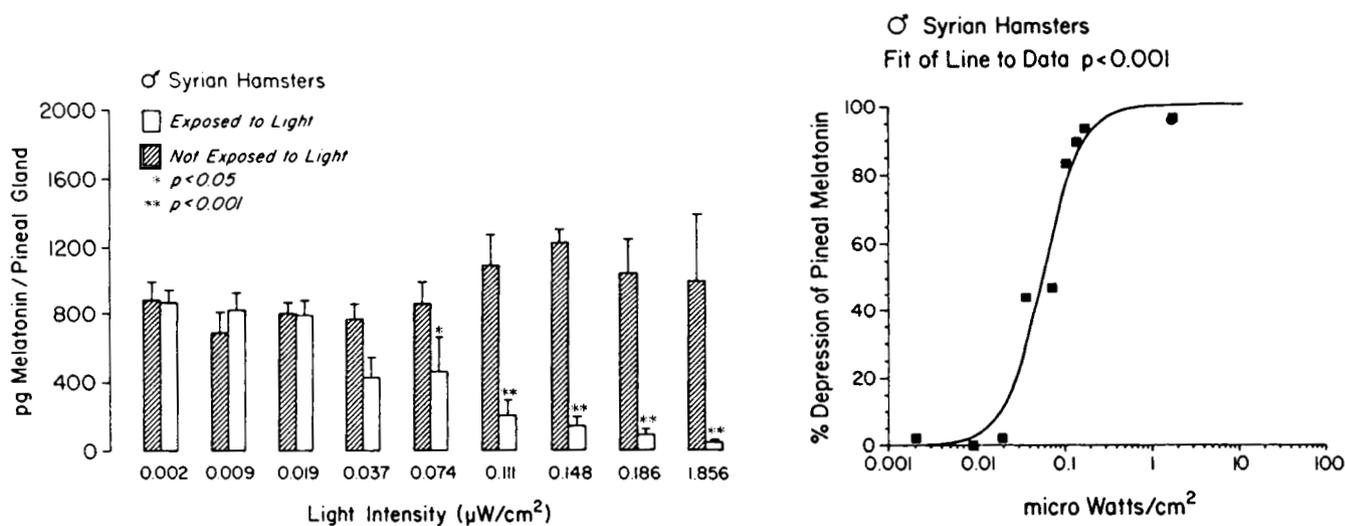


Figure 4: Fluence-response curve for melatonin suppression with cool white fluorescent light (8).

These data have important implications for the housing of animals aboard spacecraft. The inadvertant exposure of animals to white light during the night can have a strong neuroendocrine

impact. Such exposure could come from stray light leaking into the animal quarters from instrument panels, equipment, or different compartments of the capsule. Alternatively, deliberate astronaut observation of the individual animals or general animal quarters could expose animals to bright mid-deck illumination. Astronauts, in fact, prefer to periodically check the animals' status whether or not it is experimentally required. To avoid perturbing the neuroendocrine system with stray light, animal quarters should be carefully dark-proofed. In addition, animal quarters require observation ports which will allow astronauts to check animals at any time without compromising the nocturnal dark period. The specific construction of these observation ports will be discussed after review of the following data.

EFFECT OF WAVELENGTH ON MELATONIN

Studies were done on how specific spectral components interact with the neuroendocrine system (7). Animals were exposed to balanced irradiances of five different wavelength conditions for 20 minutes during the night. In one study, animals exposed to 0.2 uW/cm^2 of either red, yellow or near-ultraviolet (UV-A) light exhibited no significant suppression of melatonin. In contrast, animals exposed to 0.2 uW/cm^2 of blue or green light exhibited a very profound melatonin suppression (Figure 5). Subsequent studies showed that blue light (half-peak bandwidth range of 435 to 500 nm) was always about 25% more effective than green light (510-550 nm, half-peak bandwidth) for suppression of melatonin (7).

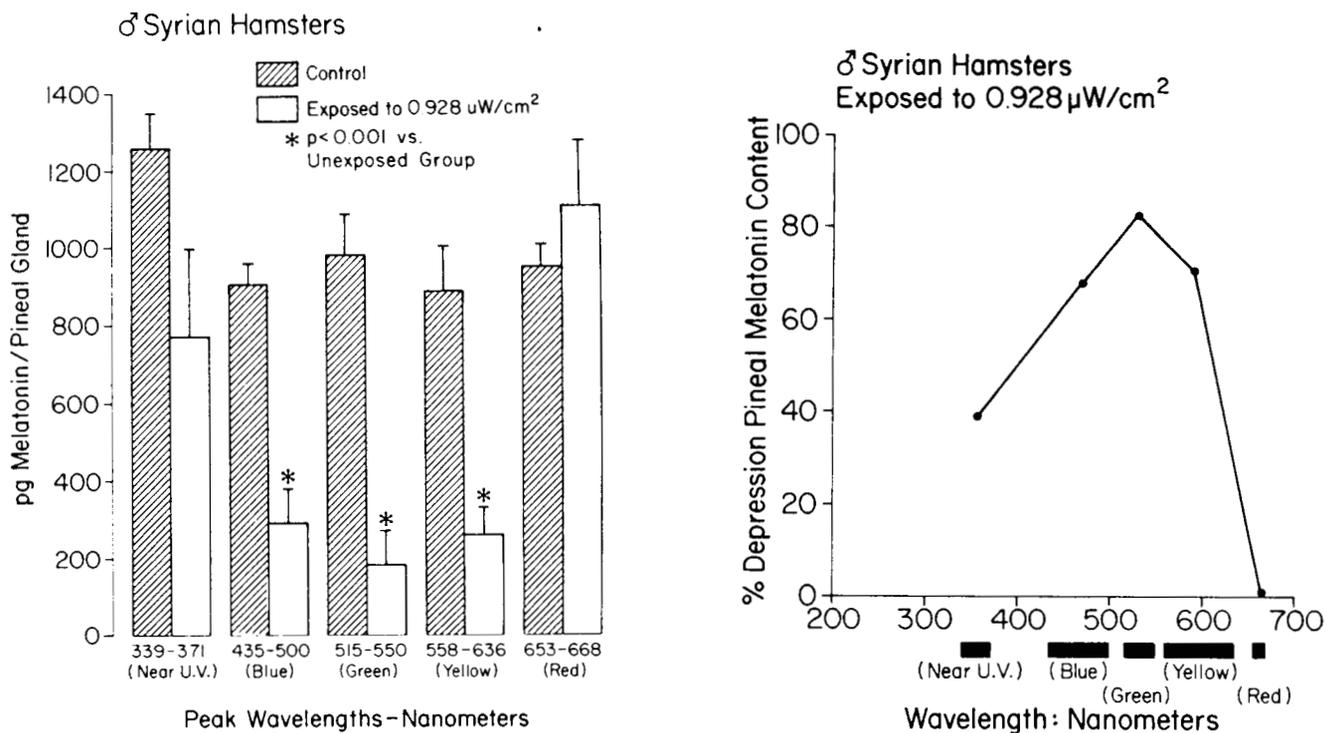


Figure 5: Effects of different light spectra on melatonin suppression (7).

All photobiological events are initiated by the absorption of a photon by a photopigment. The photopigments responsible for relaying photic information to the mammalian pineal gland have yet to be identified. Some investigators have hypothesized that rhodopsin is the primary photopigment for transducing light information to the neuroendocrine and circadian systems of rodents (15,18,19,44,49). While the weight of evidence supports this hypothesis, it is far from absolute confirmation. Additional or alternative photopigments which may participate in the photic control of the neuroendocrine system include rhodopsin-like molecules, cyanolabe (a blue-sensitive photopigment) or a specific ultraviolet sensitive photopigment (15,16,17). It should be noted that there is a species diversity

among cold-blooded vertebrates relative to putative photopigments mediating neuroendocrine responses (29). Mammals exhibit tremendous diversity in retinal structure and function (40), and thus great caution should be exercised in assuming that different mammalian species will have identical photoreceptors and photopigments for neuroendocrine regulation.

Studies with different light spectra can provide information about what photopigment or chromophore is cueing the neuroendocrine and circadian systems to light stimuli in the environment. However, it is important to remember that mammalian physiology in a natural environment is not regulated by isolated wavelengths but is controlled instead by a combination of wavelengths.

EFFECTS OF LIGHT IRRADIANCE AND WAVELENGTH ON REPRODUCTION

The hamster is a seasonal breeder. Consequently, the animal is reproductively inactive during the short days of winter. In males, short photoperiods induce decreases in testicular size, testosterone secretion, and pituitary reproductive hormones (38). As the days get longer, hamsters are spontaneously released from the gonadal quiescent stage, and become reproductively active. In the longer photoperiods of late spring, summer and early fall months, hamsters breed and have litters of pups. This seasonal cycle of reproductive activity is primarily controlled by photoperiod length (38).

Reproductive responsiveness to photoperiod length permits testing the effects of different light irradiances and

wavelengths on the reproductive axis (3,12,13). In these studies, groups of hamsters were housed in well ventilated light-proof cabinets with automatically controlled light cycles. Table I summarizes three experiments and the results of housing hamsters in experimentally modulated photoperiods for 3 months.

In each experiment, animals exposed to long photoperiods had large, reproductively active testes. Conversely, hamsters exposed to short photoperiods had significantly reduced testicular weights.

TABLE 1

| PHOTOPERIOD 400 uW/cm ² WHITE LIGHT | LENGTH & TIME OF TEST LIGHT (HOURS) | TEST LIGHT PARAMETERS (uW/cm ²) | REPRODUCTIVE STATUS AFTER 90 DAYS |
|--|---|---|---|
| EXPERIMENT 1 | | | |
| Long LD 14:10 | -- | -- | Active |
| Short LD 10:14 | -- | -- | Regressed |
| Short LD 10:14 | 3 evening | 0.002 white | Regressed |
| Short LD 10:14 | 3 evening | 0.020 white | Regressed |
| Short LD 10:14 | 3 evening | 0.200 white | Active |
| Short LD 10:14 | 3 evening | 2.000 white | Active |
| Short LD 10:14 | 3 evening | 20.00 white | Active |
| EXPERIMENT 2 | | | |
| Long LD 14:10 | -- | -- | Active |
| Short LD 10:14 | -- | -- | Regressed |
| Short LD 10:14 | 3 evening | 0.20 UV-A | Active |
| Short LD 10:14 | 3 evening | 0.20 Blue | Active |
| Short LD 10:14 | 3 evening | 0.20 Green | Active |
| Short LD 10:14 | 3 evening | 0.20 Yellow | Regressed |
| Short LD 10:14 | 3 evening | 0.20 Red | Regressed |
| EXPERIMENT 3 | | | |
| Long LD 14:10 | -- | -- | Active |
| Short LD 10:14 | -- | -- | Regressed |
| Short LD 10:14 | 1 midnight | 0.20 UV-A | Active |
| Short LD 10:14 | 1 midnight | 0.20 Blue | Active |
| Short LD 10:14 | 1 midnight | 0.20 Green | Active |
| Short LD 10:14 | 1 midnight | 0.20 Yellow | Regressed |
| Short LD 10:14 | 1 midnight | 0.20 Red | Regressed |

MID-DARK LIGHT PULSE
 ♂ SYRIAN HAMSTERS
 • p<0.001 vs. Group A

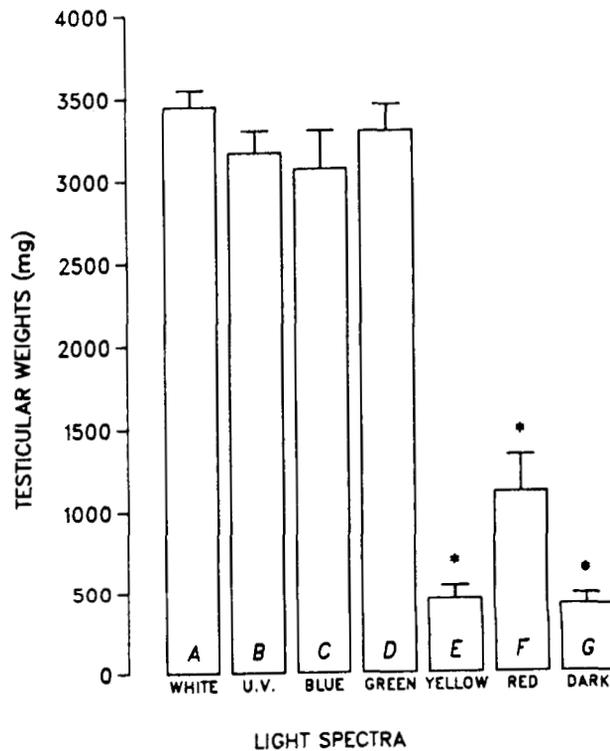


Figure 6: Reproductive effects of different light wavelengths (13).

The first reproductive study (Experiment 1) showed that extending a short photoperiod by 3 hours of dim white light (0.2 uW/cm² or higher) prevents the reproductive collapse in hamsters. In contrast, extending a short photoperiod by 3 hours of 0.02 uW/cm² or less does not block gonadal regression in hamsters (13). The second reproductive study demonstrated that extending a short photoperiod with 3 hours of dim (0.2 uW/cm²) red or yellow light did not block gonadal collapse while the same irradiance of UV-A, blue or green light blocked the effects of short photoperiod (13).

In the last reproductive study, animals in short

photoperiods were exposed to a one hour pulse of 0.2 uW/cm² UV-A, blue, green, yellow or red light in the middle of the night (3,12). As in Experiment 2, animals exposed to a pulse of UV-A, blue or green light responded similarly to animals in long photoperiods. In contrast, animals exposed to a daily pulse of red or yellow light had reproductive parameters similar to the hamsters in a short photoperiod (see Figure 6). These studies show that light wavelength and irradiance can regulate the entire reproductive axis, including prolactin, LH, and FSH from the pituitary as well as TSH from the pituitary; T3 and T4 from the thyroid and testosterone from the testes (3,12,13,47). Hence, many different neuroendocrine parameters respond to the quality of the photic environment, at least in hamsters. This raises the question about the effects of light on higher orders of animals.

EFFECTS OF LIGHT IRRADIANCE AND WAVELENGTH ON PRIMATE MELATONIN

The following preliminary data are from ongoing experiments with adult male *Cynomolgus* monkeys Macaca fascicularis (9,14,15). As in the rat, light enters the primate's eyes, stimulates the retina and sends signals by way of the retinohypothalamic pathway to the SCN. Signals are then sent from the SCN by the multisynaptic indirect pathway to the pineal gland to regulate the circadian production of melatonin. Using a modification of the model developed by Perlow, Reppert and colleagues (33,34,39), a thin cannula was inserted through a lumbar puncture into the monkey's cerebral spinal fluid (CSF) and advanced up the spinal column to the cisterna magna at the base of the brain. The

animals were then placed inside of a chamber with its own light-dark cycle and a secondary experimental monochromatic light source. The cannula was attached to a peristaltic pump which collected CSF at a very slow rate onto a fraction collector for periods up to 72 hours.

Normal melatonin rhythms from one monkey are shown in Figure 7. This animal was maintained in a white light photoperiod of 200 $\mu\text{W}/\text{cm}^2$. The scotopic phase of this animal's lighting cycle was illuminated consistently by dim red light (Kodak 1A safelight, 15W) at 3.30 $\mu\text{W}/\text{cm}^2$ or 3.5 lux. Similar data gathered from 4 monkeys showed no significant differences in melatonin rhythms with or without constant dim red light at night (14).

RED LIGHT ADAPTATION

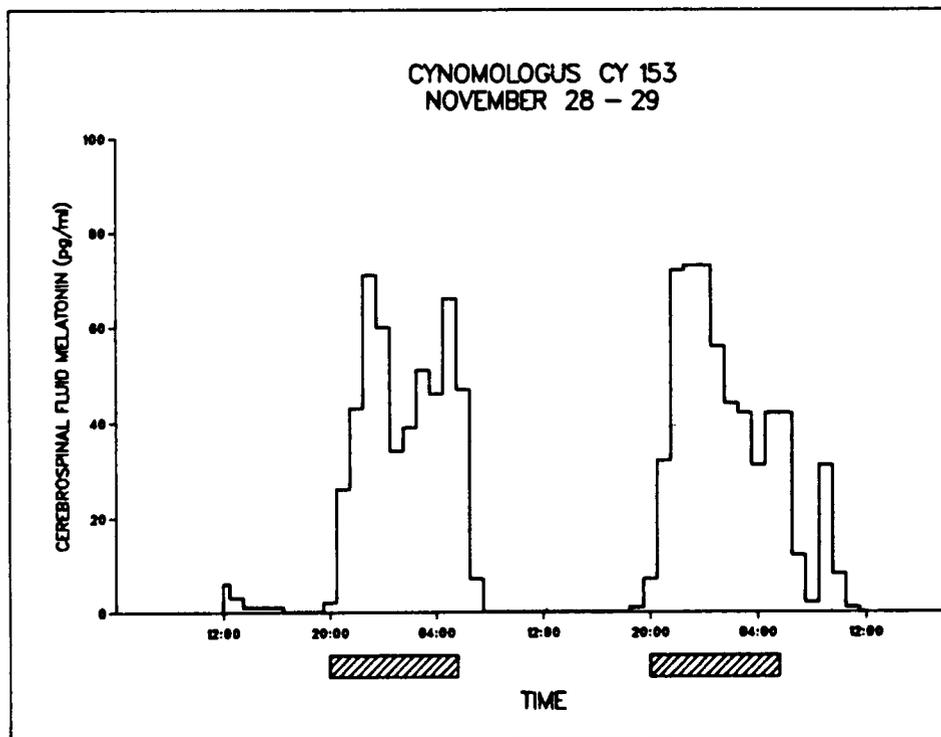


Figure 7: Normal CSF melatonin rhythm in a monkey adapted to red light at night (14).

In the first experiments, animals were exposed to a one hour pulse of 1400 uW/cm² of white light during the night. A bright white light pulse in the middle of the night caused a corresponding rapid suppression of melatonin. Similarly, Figure 8 shows that a less intense pulse of white light (16 to 20 uW/cm² which is much dimmer than ordinary room light) also suppressed melatonin in primates (9).

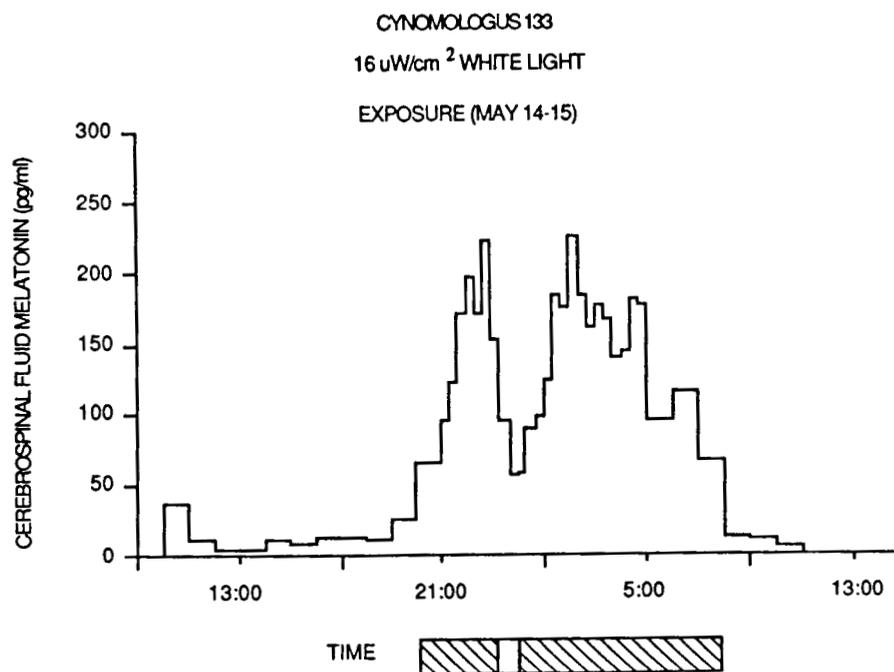


Figure 8: Melatonin suppression in monkeys by white light (9).

In other experiments, monkeys were exposed to monochromatic light during the night (9). This photic stimulus was produced by a collimated beam from a tungsten source with neutral density and interference filters to control intensity and wavelength. The data in these studies are relatively incomplete. However, there have been some surprising results to date. As shown in Figure 9, a 60 minute bright monochromatic stimulus (12 uW/cm² of 500 nm

light, 10 nm half-peak bandwidth) produced only a slight perturbation of nocturnal melatonin levels. For some monkeys, even higher irradiances of 500 nm light (60-65 $\mu\text{W}/\text{cm}^2$) produced little melatonin suppression. These data were disturbing since earlier studies on humans showed that 13 $\mu\text{W}/\text{cm}^2$ of monochromatic light at 509 nm produced an average 60% suppression of melatonin (4,51). The human studies showed that melatonin is suppressed in some individuals by as little as 1.6 $\mu\text{W}/\text{cm}^2$ of 509 nm (see Figure 10.) Given the human data, it was remarkable that 5 to 25 times brighter light was not suppressing melatonin in monkeys. This discrepancy was at least partially resolved when it was recalled that the volunteers in the human studies had their pupils fully dilated during the nighttime light exposures.

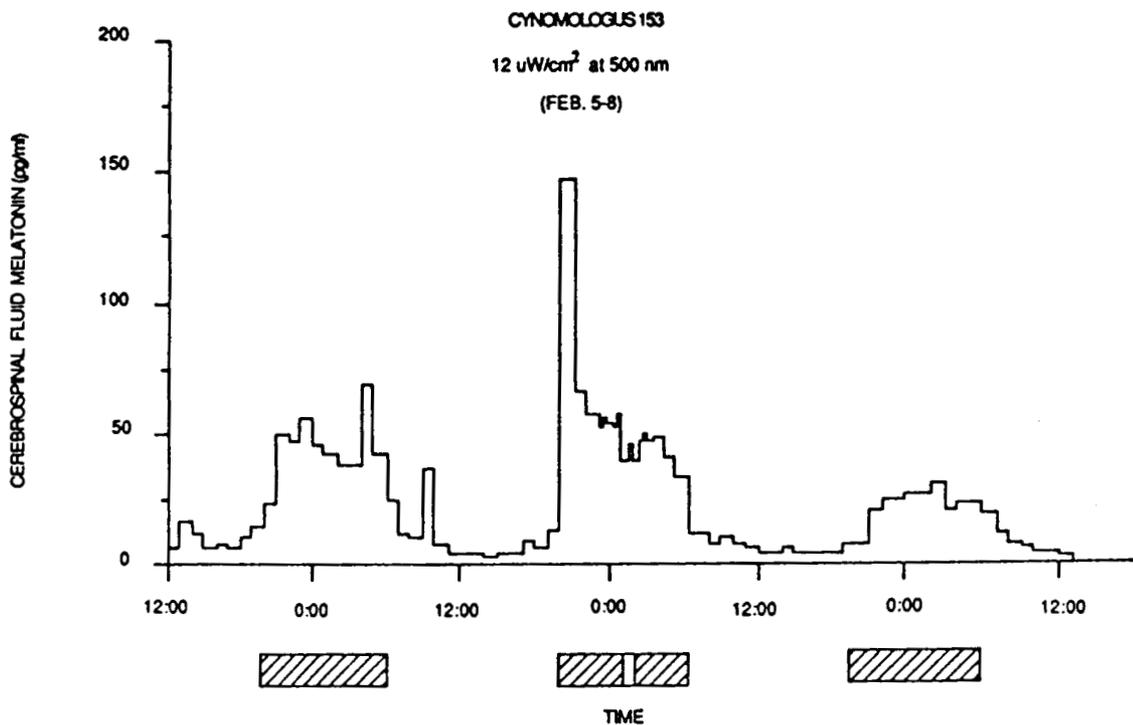


Figure 9: Melatonin rhythms of monkey exposed to bright monochromatic light on day #2. Note relatively minor suppression of melatonin after light exposure (9).

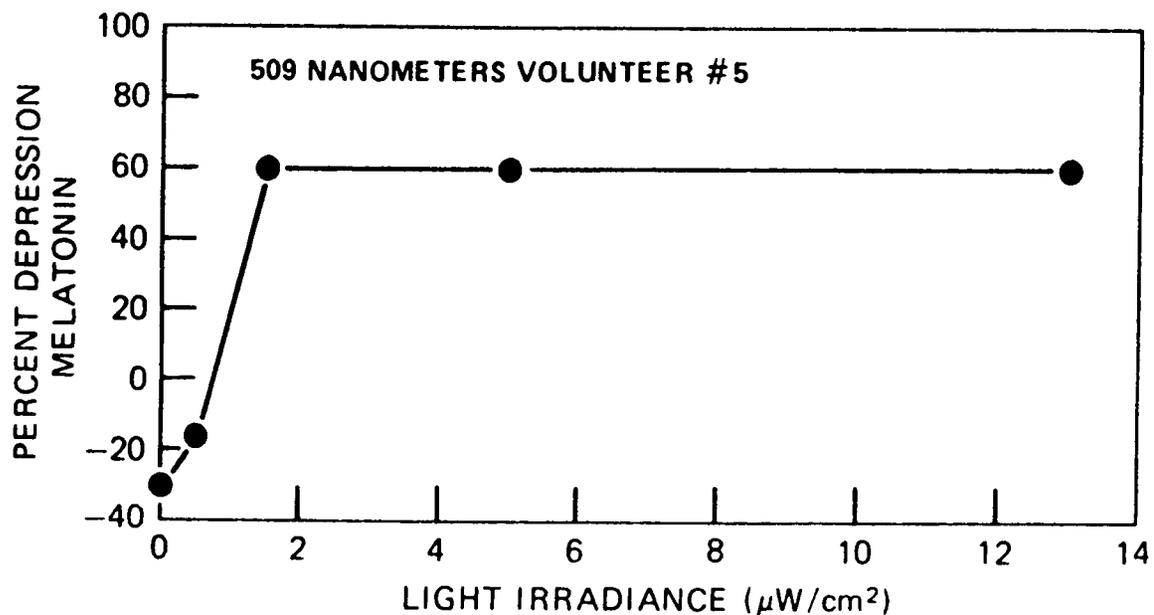


Figure 10: Fluence-response curve for monochromatic light suppression of melatonin in a normal male human (4,51).

Subsequent studies on two monkeys (9) showed that dilation of the pupils with a mydriatic agent allowed much lower intensity monochromatic light to suppress melatonin (see Figure 11). Hence, the state of pupillary dilation is an important factor in photic stimulation of the neuroendocrine system of higher species. This is a point neuroendocrinologists and circadian physiologists often forget. The eye is not a mere sensory epithelium: it is a sensory epithelium behind a window. The relative state of that window, in terms of pupil dilation and transmission of wavelengths through the clear ocular tissues, very much determines the humans' and animals' neuroendocrine responsiveness to the photic stimuli in the environment.

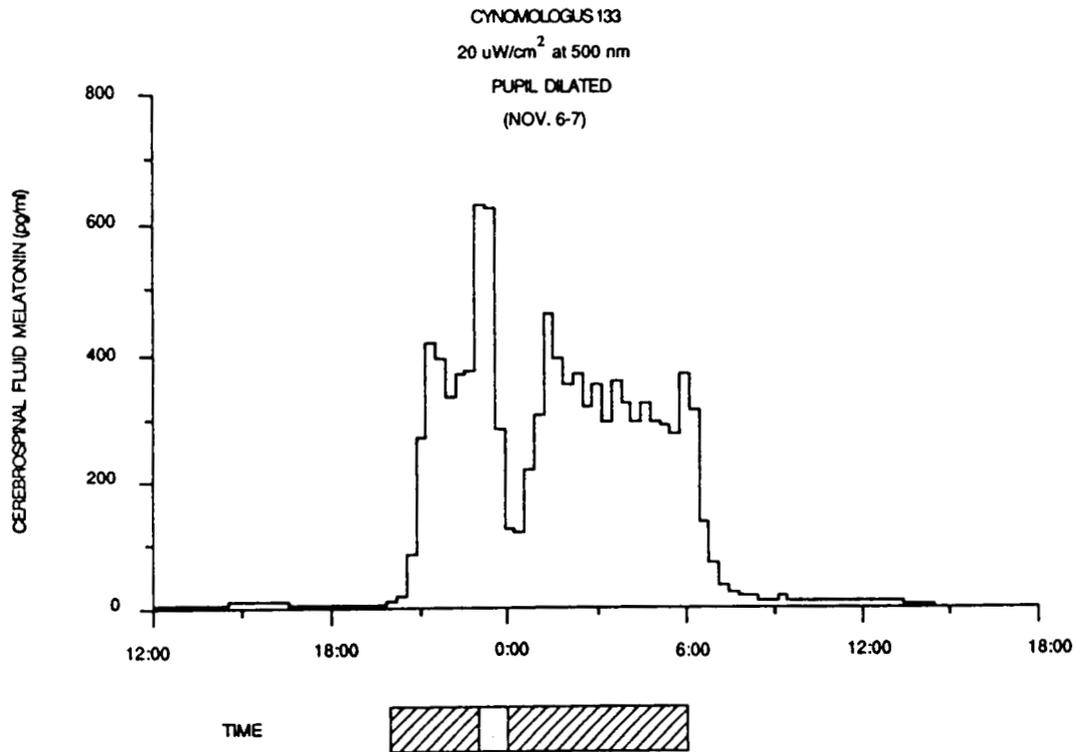


Figure 11: Monochromatic light suppression in a monkey with its pupils dilates (9).

Studies with humans and monkeys lead to several conclusions pertinent to housing of primates in spacecraft. First, inadvertant exposure of primates to light during the night can strongly perturb the primate neuroendocrine system. Therefore, stray nocturnal illumination due to light leaks or exposure to mid-deck lighting when an astronaut observes the animals should be scrupulously avoided. In general, primates are not as exquisitely sensitive to light at night as rodents. However, individual animals may have surprising responsiveness to very weak stimuli. Precautionary dark-proofing of animal quarters is an important design feature of primate housing on space vehicles. Furthermore, the astronauts should have a means for routine observation without altering the animals' neuroendocrine status.

Several design possibilities can permit astronauts to observe animals at night without disrupting the neuroendocrine system. In terms of hormonal regulation, both rodents and primates have low sensitivity to red light greater than 620 nm. Hence, astronauts could watch animals through an observation port with a cut-off filter that blocks all wavelengths below 620 nm. The cage interior could be illuminated during the night with dim red light at wavelengths above 620 nm. In rodent studies described above (7), neither 0.20 or 0.93 $\mu\text{W}/\text{cm}^2$ of red light (653-668 nm half-peak bandwidth) altered melatonin production. Animals are clearly visible under 0.93 $\mu\text{W}/\text{cm}^2$ of red illumination. Similarly, preliminary primate studies indicate that continuous nocturnal illumination of monkeys with dim red light does not alter neuroendocrine function.

There are reasons to be cautious of using red light at night in animal quarters. Most neuroendocrine and circadian studies show that rodents are insensitive to red light at night. However, Vanacek and Illnerova have shown that red light at night (5.0-200 lux) suppresses pineal N-acetyltransferase activity (46). This result was obtained with a red light source which emitted less than 5% of its illumination below 600 nm. In a different study, McCormick and Songtang (28) showed that 0.003 to 12.5 $\mu\text{W}/\text{cm}^2$ of red fluorescent light can entrain circadian behavior of rats. The authors state that less than 1% of the light in this study was below 600 nm. However, the data clearly demonstrate that red light should be used carefully and sparingly as a routine component of animal quarters in space. Further

experiments are clearly warranted to determine the optimum irradiances and wavelengths of red light for observing animals without disturbing their physiology.

An alternative system for animal observation at night could be based on infra-red viewing instruments. With this technology, the animal cage is illuminated exclusively with weak infra-red energy. Astronauts would then use an observation port that detects reflected infra-red energy and converts it to a visible image. This approach would avoid using any visible light energy in animal compartments at night. Military infra-red night vision equipment could be adapted to this purpose as well as commercially available infra-red equipment. Although an infra-red system has less chance of affecting animal physiology, this technology is more complex (and thus more liable to breakdown) and requires additional energy to operate.

EFFECTS OF ULTRAVIOLET RADIATION ON THE NEUROENDOCRINE SYSTEM

Visible light (400-760 nm) perceived by the eyes regulates the neuroendocrine system in mammals. In contrast, it has been nearly axiomatic that the mammalian eye and, consequently, the neuroendocrine system are insensitive to ultraviolet radiation. Photobiologists divide ultraviolet radiation into three bandwidths: near ultraviolet (UV-A, 400-320 nm): middle-ultraviolet UV-B, 320-280 nm): and far-ultraviolet (UV-C, 280-200 nm) (32). This section concerns the effects of UV-A on the neuroendocrine systems of rats, hamsters and mice (2,10,12,13,15,16,35).

For visible or ultraviolet radiation to stimulate the retina and activate the visual or neuroendocrine systems, it must be transmitted through the clear ocular media. The cornea, aqueous humor and vitreous humor each transmit visible light and ultraviolet wavelengths down to 295 nm (21,25,32). It is often thought that the crystalline lens acts as a selective filter which does not transmit wavelengths below 400 nm. Whereas this is true for adult humans (21,25,32), it is not the case in Long Evans Hooded rats (Rattus norvegicus), Syrian hamsters (Mesocricetus auratus), or wild captured mice (Peromyscus leucopus). The lens of the rat (Figure 12), hamster and mouse each transmits ultraviolet radiation down to 300 nm (10,16).

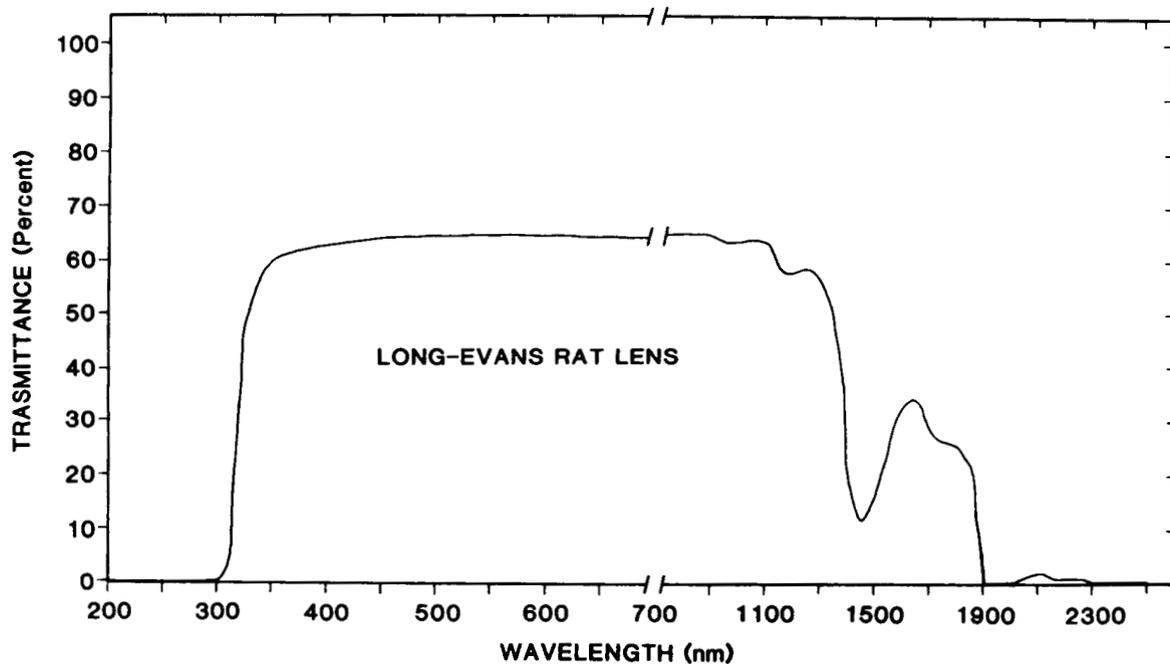


Figure 12: Transmission characteristics of a rat lens (10).

Given that UV-A wavelengths reach the retina through the clear ocular media, it was logical to test the capacity of this

energy for regulating neuroendocrine parameters. Rats were exposed to a nocturnal pulse of 0.5 or 1.5 uW/cm² (2.72×10^{14} or 8.16×10^{14} photons/cm², respectively) of monochromatic UV-A (360 nm, 10 nm half-peak bandwidth). Hence, monochromatic radiation (UV-A, 360 nm) outside of the "visible" spectrum can suppress NAT melatonin forming enzyme (Figure 13).

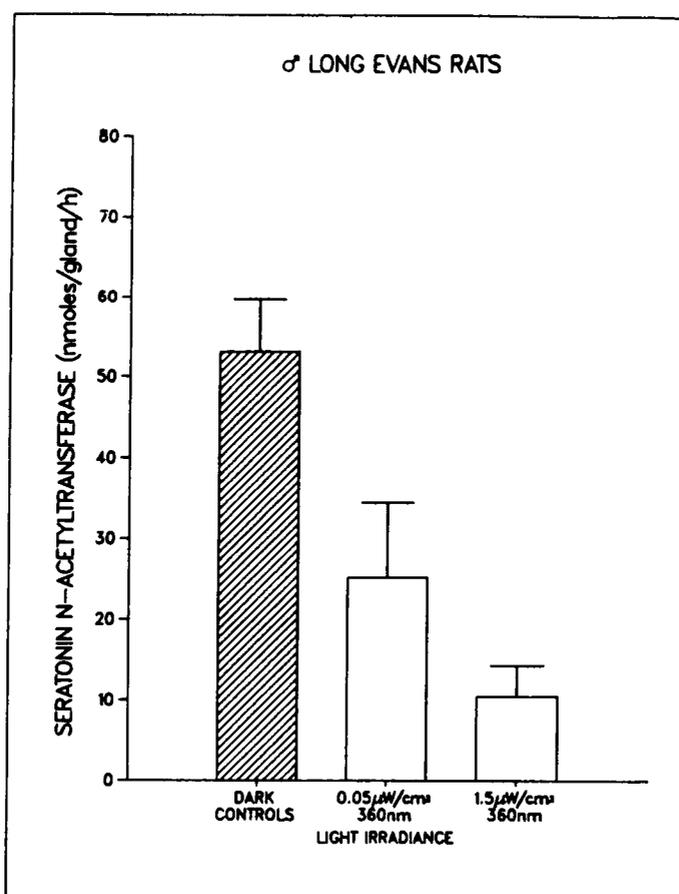


Figure 13: Suppression of pineal NAT by monochromatic UV-A in the rat (10).

The influence of UV-A wavelengths on the neuroendocrine system is not limited to rats. Studies have demonstrated that exposure of Syrian hamsters to broad-band (340-385 nm, half-peak bandwidth) and monochromatic (360 nm) UV-A during the night can suppress pineal melatonin (16,35). Two fluence-response curves

for the suppression of pineal melatonin by monochromatic UV-A (360 nm) and visible light (500 nm) are shown in Figure 14. These data show that hamsters are ten times more sensitive to 500 nm light versus 360 nm radiation. However, this difference in sensitivity should not obscure the fact that remarkably low levels of UV-A can induce a 90% suppression of melatonin.

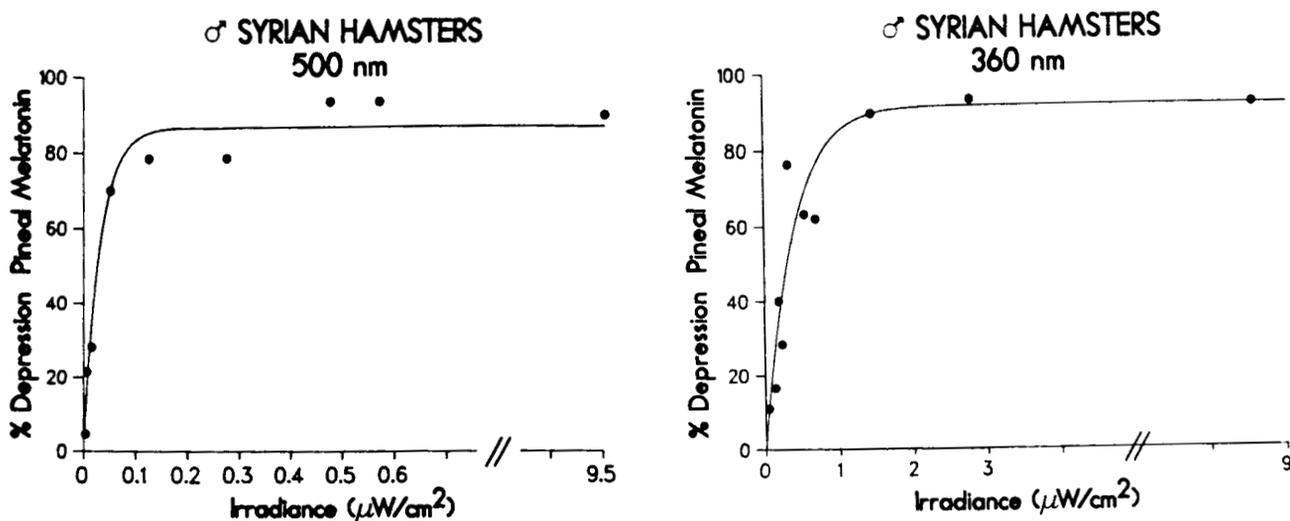


Figure 14: Fluence-response curves for melatonin suppression by monochromatic visible and ultraviolet radiation in hamsters (35).

In addition to melatonin suppression, long term exposure to broad bandwidths of UV-A can influence the reproductive axis of Syrian hamsters as shown in the studies described above (3,12,13). Adding a one hour pulse of 0.2 $\mu\text{W}/\text{cm}^2$ UV-A in the middle of the night or three hour evening pulse of 0.2 $\mu\text{W}/\text{cm}^2$ UV-A in the evening, blocks the short photoperiod-induced reproductive system collapse (Figure 6).

After establishing that UV-A modulates neuroendocrine

responses in two inbred laboratory species, it seemed appropriate to test the effects of UV-A on a wild rodent species (2). Genetically heterogenous adult Peromyscus leucopus were derived from a colony of animals captured in Connecticut for the studies shown in Figure 15. In those studies, mice were exposed to a 5 minute pulse of equal photon densities (2.64×10^{15} photons/cm² of monochromatic 320 nm, 340 nm or 360 nm UV-A during the night. Additional groups of mice were exposed to equal photon densities of 500 nm, 560 nm and 640 nm light during the night. As shown in Figure 15, each UV-A stimulus as well as the 500 nm and 560 nm stimuli significantly suppressed melatonin whereas an equal photon density of 640 nm did not (2). The physical parameters of the photic stimuli in this experiment are shown in Table II.

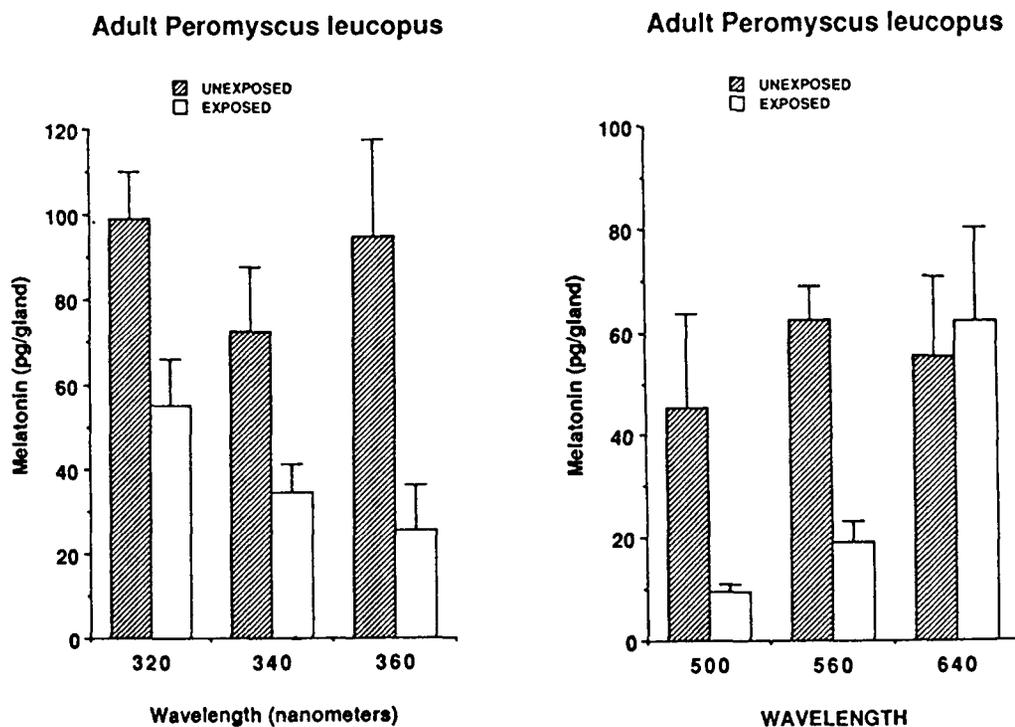


Figure 15: Suppression of melatonin by monochromatic wavelengths of visible and ultraviolet radiation in mice (2).

TABLE II

Radiometric and photometric measurements of the balanced photon exposures for each trial(2)

| WAVELENGTH | PHOTONS/CM ² | uW/cm ² | LUX |
|------------|-------------------------|--------------------|-------|
| 320 | 2.64 x 10 ¹⁵ | 5.46 | 0.00 |
| 340 | 2.64 x 10 ¹⁵ | 5.14 | 0.00 |
| 360 | 2.64 x 10 ¹⁵ | 4.86 | 0.00 |
| 500 | 2.64 x 10 ¹⁵ | 3.49 | 7.70 |
| 560 | 2.64 x 10 ¹⁵ | 3.12 | 21.20 |
| 640 | 2.64 x 10 ¹⁵ | 2.73 | 3.26 |

The studies above show that UV-A must be considered an effective stimulus to the neuroendocrine systems of at least three rodent species. This represents a significant extension of what the neuroendocrine system treats as "visible" radiation. Very minor amounts of UV-A are able to have profound hormonal consequences (2,10,12,13,15,16,35).

These data also show that photometric values (lux, footcandles, lumens) are inadequate for describing the photic environment of these species. In radiometric terms, 0.10 to 3.75 uW/cm² of UV-A can cause major neuroendocrine changes. However, this photic stimulus will register 0.00 lux, 0.00 footcandles or 0.00 lumens on standard photometers. Photometric measurements such as lux, footcandles and lumens are based exclusively on the photic responsiveness of the human eye during bright daylight. Use of photopic meters is not appropriate

unless the specific photopigments regulating human daytime vision are responsible for mediating the rodent and primate neuroendocrine system. For convenience of relating data from laboratories that have not yet converted to radiometric instrumentation, it is useful to include photopic conversions of light energy levels as in Table III.

TABLE III

| <u>BROADBAND SOURCES</u> | | | <u>MONOCHROMATIC SOURCES</u> | | |
|--------------------------|---|------------|------------------------------|---|------------|
| <u>TYPE</u> | <u>$\mu\text{W}/\text{cm}^2$</u> | <u>LUX</u> | <u>TYPE</u> | <u>$\mu\text{W}/\text{cm}^2$</u> | <u>LUX</u> |
| Sunlight | 55,000 | 110,000 | 640 nm | 2.73 | 3.26 |
| Sunlight twilight | 3.5 | 10 | 560 nm | 3.12 | 21.20 |
| Fluorescent cool white | 928 | 3,120 | 509 nm | 13 | 42.94 |
| Fluorescent cool white | 400 | 1,344 | 509 nm | 1.6 | 5.28 |
| Fluorescent cool white | 200 | 672 | 500 nm | 65 | 143.39 |
| Fluorescent cool white | 1.856 | 6.236 | 500 nm | 60 | 132.36 |
| Fluorescent cool white | 0.186 | 0.624 | 500 nm | 12 | 26.47 |
| Fluorescent cool white | 0.148 | 0.497 | 500 nm | 3.49 | 7.70 |
| Fluorescent cool white | 0.111 | 0.373 | 360 nm | 4.86 | 0.00 |
| Fluorescent cool white | 0.074 | 0.249 | 360 nm | 1.50 | 0.00 |
| Fluorescent cool white | 0.037 | 0.124 | 360 nm | 0.50 | 0.00 |
| Fluorescent cool white | 0.019 | 0.064 | 340 nm | 5.14 | 0.00 |
| Fluorescent cool white | 0.009 | 0.030 | 320 nm | 5.46 | 0.00 |
| Fluorescent cool white | 0.002 | 0.007 | | | |
| Fluorescent red | 0.930 | 1.007 | | | |
| Fluorescent red | 0.200 | 0.216 | | | |
| Fluorescent yellow | 0.200 | 0.358 | | | |
| Fluorescent green | 0.200 | 1.121 | | | |
| Fluorescent blue | 0.200 | 0.029 | | | |
| Fluorescent UV-A | 0.200 | 0.00 | | | |
| Incandescent white | 1400 | 3760 | | | |
| Incandescent white | 20 | 53.80 | | | |
| Incandescent red | 3.3 | 3.50 | | | |

Physiologically, UV-A appears to be an important environmental stimulus to the neuroendocrine system. Irradiances of UV-A that regulate the pineal gland are at least 10,000-fold lower than UV-A at the earth's surface during full sunlight (45) and at least 10-fold lower than UV-A twenty minutes after sunset. Both rodents and primate species exhibit activity during full sunlight, dusk and dawn periods.

The data on UV-A interactions with the neuroendocrine system are important to consider in terms of design of daytime lighting in animal compartments. Natural animal habitats have a UV-A component even when the species is primarily active at night. Since UV-A can regulate normal neuroendocrine responses, it is logical to include these wavelengths in the daily environment that animals inhabit.

Concerning the lamps used for the daylight illumination of animal quarters in space, it seems appropriate to use a white light source which mimics the natural solar spectrum. Animals evolved in environments illuminated predominantly by sunlight. The above studies on UV-A illustrate that non-visible radiation between 320-400 nm participates in the normal regulation of the neuroendocrine system in rodents at least. Hence, including these wavelengths in the daily environment is appropriate.

CONCLUSION

In designing the illumination of animal quarters in space laboratories, a variety of physiological systems must be considered. In addition to the visual system, dermal physiology, circadian rhythms and the neuroendocrine system respond to photic input. Hence, animal illumination must provide adequate photic stimulation for the normal regulation of each biological system. As shown above, the visual system of rodents and primates respond to different intensity threshold levels than the neuroendocrine system. Thus, a light intensity which supports vision may not optimally regulate the neuroendocrine axis. Sufficient light must be provided which regulates all photic responding systems. However, it must be recalled that both visible light and ultraviolet radiation can be toxic when exposure levels are too high (20,48). Excessive photic stimuli can cause skin lesions, corneal burns, lens cataracts and retinal damage. Hence, animal quarters must be illuminated with light intensities that exceed the threshold for visual and biological regulation, but which are below thresholds for toxicity. Unfortunately, all intensity thresholds and wavelength characteristics for regulation and toxicity are not yet known.

The current deficits in our understanding of mammalian photobiology introduce elements of uncertainty in designing lighting for animals aboard spacecraft. Thus, animal lighting should be designed so that it can be easily modified. Ideally, the light source itself should emit visible (400-800 nm), near-ultraviolet (320-400 nm) and middle-ultraviolet (290-320 nm)

wavelengths in a balance relatively similar to sunlight on the planet's surface. The source of its ballast or socket should be placed so that neutral density and/or wavelength cut-off filters can be placed between the animal and the bulb. Thick glass filters (2-8 mm each) or very thin plastic filter, (less than 0.1 mm each) for modifying light intensity and wavelength are currently available. A modifiable filtering system permits flexibility for changing light characteristics for different species or experimental requirements. Such a filtering system also allows the lighting system to be modified as new discoveries are made concerning photic regulation and light toxicity.

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LIGHTING FOR RODENTS ON SPACELAB:
CONSIDERATIONS OF LIGHT-TOXICITY

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It is a pleasure to attend this exciting workshop and to hear so many interesting papers in fields that I ordinarily do not have the chance to hear. My report deals with a topic that is quite different from all the others which have been presented so far.

I shall describe a phenomenon called "retinal light-damage". Some twenty years ago it was reported that moderate levels of visible light can be toxic to the rat retina, especially if the light is continuously presented. Retinal light-damage is now well-documented and the following is just a partial list of parameters, known to affect the susceptibility to damage in rats.

-Intensity and time of the exposure. However, no solid evidence exists that I and t are reciprocally related as they are in the typical Bunsen-Roscoe Law situation. (1, 2)

-The age of the animal. Older animals are generally more susceptible than are young ones. (1, 3, 4)

-Ocular pigmentation. Melanin in pigment epithelium and in the choroid protects against damage to some extent but protection is mainly afforded by a pigmented iris which simply reduces the retinal exposure. (5, 6, 7)

-Wavelength of the light. The reported action spectra for damaging the retina match the absorption spectra of rod visual pigment. (1, 8)

-Body temperature. Elevated body temperature enhances the damage for a given exposure. (1)

-Hormonal status of the animal. Stress and sexual maturity, with their attendant hormonal changes, bias the retina toward damage. (3, 9)

-Antioxidant status of the retina. Evidence exists that at least some of the photoreceptors which die might have otherwise been spared if antioxidant levels were elevated. (10, 11)

-Light history. Previous exposure to near-damaging intensities has the effect of protecting against damage in subsequent exposures. On the other hand, animals raised in very dim light (or in darkness) are highly susceptible if exposed to lights which are brighter than those normally encountered. (1, 12)

-Optic nerve effects. The two eyes of an animal with unilateral optic nerve section damage unequally: the eye with the sectioned nerve damages less than the other eye in the same animal. (13)

Although the above summary derives from work on rats, it is now clear that many species can suffer retinal light damage; indeed, a list of said species would include entries as diverse as pigeons and monkeys. This paper deals exclusively with albino rats.

The majority of presentations at this workshop have dealt with circadian effects of light and, especially, with the threshold levels needed to entrain the rhythms. In other words, the concern has mainly been, "How dim can the cyclic light be and still result in normal physiology?" As you might guess, my paper deals with the converse problem: "What is the upper limit of intensity beyond which retinal damage will occur?" Between those two limits there should be an intensity range that will be "safe", i.e. will result in normal physiology and undamaged retinas.

The answer from our experiments on albino rats is not simple but for practical purposes it translates as "between 10 and 100 lux, cycled 12/12". However, there are several caveats which must be seriously considered before applying this guideline.

-The main concern is that the light-history of the animals must be taken into account. For example, adult albino rats, born and raised in 3 lux cyclic, will suffer retinal damage if put into very moderate (e.g. 133 lux) cyclic light (14). Therefore, my suggestion is that the rats should be born and raised at whatever intensity is to be used in the experimental setting. The LD periods should be invariant as well.

-Care should be taken to avoid high body temperatures. This is especially true while the lights are on.

-Conversely, low temperature is stressful to rats and this could produce a susceptibility to retinal damage even in an otherwise non-damaging intensity.

In summary, I have enumerated factors which are known to affect retinal light-damage susceptibility. After consideration of these factors, it seems that 10-100 lux, cycled 12-12, might meet the guidelines to avoid retinal light damage. However, because no simple answer exists in these matters, certain precautions are also suggested.

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APPENDIX A
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| 16. Abstract A workshop, sponsored by Ames Research Center, was held at San Jose State University, San Jose, California, July 16-17, 1987, to discuss and correlate observations and theories relating to lighting requirements in animal habitats for rodents and nonhuman primates in microgravity (near space). This volume represents the results of that workshop. It contains a summary of the conclusions reached and recommendations for lighting animal housing modules used in microgravity related projects. The recommendations cover various aspects of habitat lighting including engineering standards for intensity, spectral properties, and light cycle controls. | | | | | |
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